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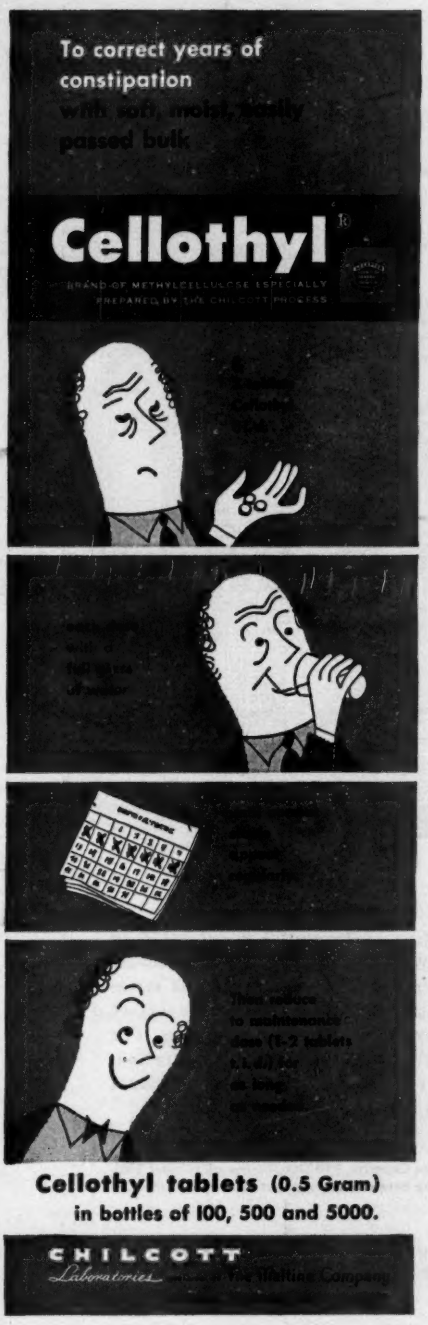
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1. Newey, J. A., and Goetzl, F. R.: Permanente Med. Bull. 7:67 (July) 1949.

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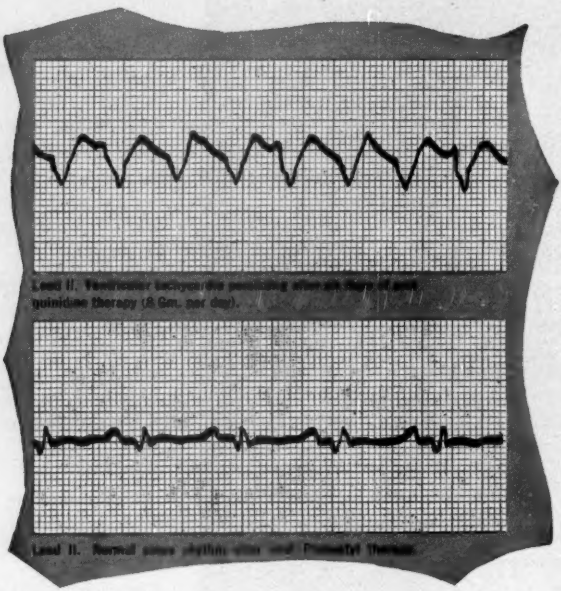

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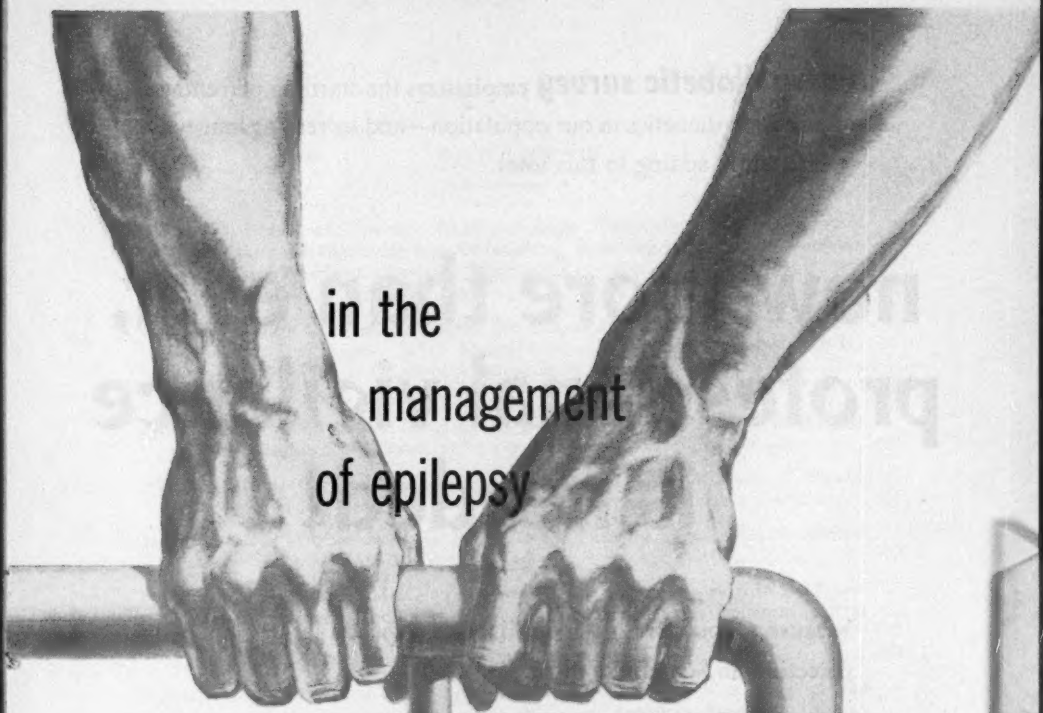

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*Cutting, W. C.: A Manual of Clinical Therapeutics, ed. 2, Philadelphia, W. B. Saunders & Co., 1948, p. 484.

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FOLLOW-UP STUDY OF THERAPY IN FORTY-EIGHT CULTURALLY PROVED CASES OF BRUCELLOSIS

Streptomycin and Sulfadiazine, Aureomycin, and Chloramphenicol (Chloromycetin®)

WESLEY W. SPINK, M.D.
WENDELL H. HALL, M.D.
AND
ROBERT MAGOFFIN, M.D.
MINNEAPOLIS

THE THERAPY of human brucellosis has been under investigation in this clinic for several years, and the results of experimental and clinical studies have been presented in a number of reports.[1] The first decisive therapeutic advancement in brucellosis was recorded by Pulaski and Amspacher,[2] who pointed out the bene-

From the Department of Medicine, University of Minnesota Hospitals, and the Veterans Administration Hospital, University of Minnesota Medical School.

Reviewed by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

1. (*a*) Hall, W. H., and Spink, W. W.: In Vitro Sensitivity of Brucella to Streptomycin: Development of Resistance During Streptomycin Treatment, Proc. Soc. Exper. Biol. & Med. **64**:403, 1947. (*b*) Hall, W. H., and Spink, W. W.: Therapy of Experimental Brucella Infection in the Developing Chick Embryo: I. Infection and Therapy Via the Allantoic Sac, J. Immunol. **59**:379, 1948. (*c*) Shaffer, J. M., and Spink, W. W.: Therapy of Experimental Brucella Infection in the Developing Chick Embryo: II. Infection and Therapy Via the Yolk Sac, ibid. **59**:393, 1948. (*d*) Therapy of Experimental Brucella Infection in the Developing Chick Embryo: III. Synergistic Action of Streptomycin and Sulfadiazine, ibid. **60**:405, 1948. (*e*) Magoffin, R.; Anderson, D., and Spink, W. W.: Therapy of Experimental Brucella Infection in the Developing Chick Embryo: IV. Therapy with Aureomycin, ibid. **62**:125, 1949. (*f*) Yow, E. M., and Spink, W. W.: Experimental Studies on the Action of Streptomycin, Aureomycin, and Chloromycetin on Brucella, J. Clin. Invest. **28**:871, 1949. (*g*) Braude, A. I., and Spink, W. W.: The Action of Aureomycin and Other Chemotherapeutic Agents in Experimental Brucellosis, J. Immunol. **65**:185, 1950. (*h*) Spink, W. W.; Hall, W. H.; Shaffer, J. M., and Braude, A. I.: Human Brucellosis: Its Specific Treatment with a Combination of Streptomycin and Sulfadiazine, J. A. M. A. **136**:382 (Feb. 7) 1948. (*i*) Spink, W. W.: Pathogenesis of Human Brucellosis with Respect to Prevention and Treatment, Ann. Int. Med. **29**:238, 1948. (*j*) Spink, W. W.; Hall, W. H.; Shaffer, J., and Braude, A. I.: Treatment of Brucellosis with Streptomycin and a Sulfonamide Drug, J. A. M. A. **139**:352 (Feb. 5) 1949. (*k*) Spink, W. W.; Braude, A. I.; Castaneda, M. R., and Silva-Goytia, R.: Aureomycin Therapy in Human Brucellosis Due to Brucella Melitensis, ibid. **138**:1145 (Dec. 18) 1948. (*e*) Braude, A. I.; Hall, W. H., and Spink, W. W.: Aureomycin Therapy in Human Brucellosis Due to Brucella Abortus, ibid. **141**:831 (Nov. 19) 1949.

2. Pulaski, E. J., and Amspacher, W. H.: Streptomycin Therapy in Brucellosis, Bull. U. S. Army M. Dept. **7**:221, 1947.

ficial effect of the simultaneous administration of streptomycin and sulfadiazine. This observation was soon confirmed by others.[3] Although the combination of streptomycin and sulfadiazine proved to be an effective form of therapy, such treatment was not without certain disadvantages. Streptomycin had to be administered parenterally, and sulfadiazine produced toxic reactions. But more disturbing were the number of infections that relapsed after a temporary period of improvement. A further advance in the therapy of human brucellosis was made with the introduction of aureomycin. Several experimental and clinical reports, in addition to those from this clinic, have demonstrated the anti-Brucella action of aureomycin.[4] Aureomycin appeared to have definite advantages over the combination of streptomycin and sulfadiazine in that aureomycin could be administered by mouth; it was without serious side effects, and many patients with serious complications showed rapid improvement with aureomycin treatment following a relapse after therapy with streptomycin and sulfadiazine. Subsequently, similar therapeutic properties were ascribed to chloramphenicol.[5]

Most investigators are agreed that use of streptomycin and sulfadiazine, aureomycin, or chloramphenicol will favorably influence the clinical course of brucellosis. The available reports have been concerned principally with the immediate outcome of the patients. Because of the insidious nature of this disease and the tendency of brucellosis to relapse, long-term follow-up studies on culturally proved cases are desirable, including clinical, serologic, and bacteriologic investigations before and after treatment. To date 80 patients with bacteriologically proved brucellosis have

3. Eisele, C. W., and McCullough, N. B.: Combined Streptomycin and Sulfadiazine Treatment in Brucellosis, J. A. M. A. **135**:1053 (Dec. 20) 1947. Morales, H. F.: The Treatment of Brucellosis with Sulfadiazine and Streptomycin, Puerto Rico J. Pub. Health **24**:362, 1948-1949. Herrell, W. E., and Nichols, D. R.: The Combined Use of Streptomycin and Sulfadiazine in the Treatment of Brucellosis, M. Clin. North America **33**:1079, 1949. Molinelli, E. A.; Ithurralde, D.; Basso, G.; Miyara, S.; Speroni, A.; Vital, V. C.; Cabassi, E., and Pandolfo, G.: Recent Developments in the Therapy of Human Brucellosis, Puerto Rico J. Pub. Health **25**:29, 1949.

4. Bryer, M. S.; Schoenbach, E. B.; Wood, R. M., and Long, P. H.: The Treatment of Acute Brucellosis with Aureomycin, Bull. Johns Hopkins Hosp. **84**:444, 1949. Debono, J. E.: Aureomycin in Undulant Fever, Lancet **2**:326, 1949. Lacy, H., and Lankford, C. E.: Comparative Bacteriostatic Effect of Aureomycin and Streptomycin for Brucella, Texas Rep. Biol. & Med. **7**:260, 1949. Cruickshank, J. C.: The Treatment of Experimental Infection with Brucella Abortus in Guinea-Pigs, Including a Trial of Aureomycin, Month. Bull. Min. Health & Pub. Health Lab. Serv. **8**:190, 1949. Lindek, E. W.: Aureomycin in Brucellosis, Brit. M. J. **1**:985, 1950. Boal, D. H., and Ishii, A. H.: Brucella Suis Infection Treated with Aureomycin: Case Report, J. Missouri M. A. **47**:364, 1950. Stoffel, E.: Aureomycin: Therapie des Morbus Bang, Schweiz. med. Wchnschr. **80**:1005, 1950.

5. Killough, J. H.; Magill, G. B., and Smith, R. C.: Terramycin, Chloramphenicol and Aureomycin in Acute Brucellosis: Preliminary Report, J. A. M. A. **145**:553 (Feb. 24) 1951. Woodward, T. E.; Smadel, J. E.; Holbrook, W. A., and Raby, W. T.: The Beneficial Effect of Chloromycetin on Brucellosis, J. Clin. Invest. **28**:968, 1949. Ralston, R. J., and Payne, E. H.: Treatment of Chronic Brucellosis with Chloramphenicol and Aureomycin, J. A. M. A. **142**:159 (Jan. 21) 1950. Harris, H. J.: Aureomycin and Chloramphenicol in Brucellosis with Special Reference to Side Effects, ibid. **142**:161 (Jan. 21) 1950. Knight, V.; Ruiz-Sanchez, F., and McDermott, W.: Chloramphenicol in the Treatment of the Acute Manifestations of Brucellosis, Am. J. M. Sc. **219**:627, 1950.

been treated under our supervision. Since 24 of the 80 patients were treated in cooperation with Dr. M. Ruiz Castaneda and his associates in Mexico,[1k] only the immediate results were reported, and these patients have been excluded from the present series. In addition, eight patients have been treated in Minneapolis too recently for the purposes of this study. Adequate follow-up studies have been carried out in 48 of the patients, and these are the basis for the present report.

MATERIAL AND METHODS OF STUDY

Causation.—An important feature in the evaluation of therapy in brucellosis is the species of Brucella causing the disease. Brucella abortus was isolated from 44 of the 48 patients, Brucella suis from two, and Brucella melitensis from two. This preponderance of cases caused by Br. abortus emphasizes the fact that a majority of the cases of human brucellosis in Minnesota are due to this species.

Sex Distribution.—Of the 48 patients, 42 were male and six were female. Brucellosis is primarily a disease of males, a conclusion arrived at in a study of a larger number of cases in Minnesota.[6]

Age Distribution.—The average age of the 48 patients was 31.1 yr. The youngest patients treated included two children 1½ and 3½ yr. old.

Duration of Disease.—The length of time the patients had been ill was taken to be the interval between the initial appearance of the symptoms and the beginning of chemotherapy. In accordance with conclusions reached in previous communications,[7] cases were classified as acute if the illness had persisted for three months or less, as subacute when the patients had been ill for more than three months but less than 12 mo., and as chronic when the duration was 1 yr. or more. Accordingly, there were 33 patients with acute brucellosis, 11 with subacute brucellosis, and four with chronic brucellosis.

Complications.—The majority of the patients had to be hospitalized because of the severity of their illness. The serious nature of several of the cases was reflected in the complications of brucellosis that were observed. One patient had subacute bacterial endocarditis due to Br. abortus; two had bacteremias due to Br. abortus with inactive valvular heart disease; two had a disabling neuritis and one had painful radiculitis; two had arthritis of the hip joint, and one had osteitis of the sacroiliac joint. Three patients had amebiasis in addition to brucellosis.

Serologic and Bacteriologic Studies.—Serial agglutination tests on the patients' serum were performed before and after therapy, employing an antigen of Br. abortus supplied by the Bureau of Animal Industry, United States Department of Agriculture. A standardized amount of antigen was added to twofold saline dilutions of serum and incubated in a water bath at 37 C. for 48 hr. Serial cultures of blood were made before and after therapy, utilizing the double medium of Castaneda (a mixture of bacto-tryptose, sodium chloride, sodium citrate, agar, and distilled water).[8] The isolated organisms were identified as Brucella with the aid of anti-Brucella rabbit serum. The species of Brucella were identified according to the ability of the organisms to produce hydrogen sulfide, the growth of the bacteria on dye plates, and the carbon dioxide requirements for growth.[9]

---

6. Magoffin, R. L.; Kabler, P.; Spink, W. W., and Fleming, D.: An Epidemiologic Study of Brucellosis in Minnesota, Pub. Health Rep. **64**:1021, 1949.

7. (*a*) Spink, W. W., and Magoffin, R. L.: Clinical Course of Human Brucellosis in Minnesota, in Proceedings of the Third Inter-American Congress on Brucellosis, Washington, D. C., 1950, to be published. (*b*) Spink, W. W.: What is Chronic Brucellosis? Ann. Int. Med. **35**:358, 1951.

8. Castaneda, M. R.: A Practical Method for Routine Blood Cultures in Brucellosis, Proc. Soc. Exper. Biol. & Med. **64**:114, 1947.

9. Huddleson, I. F.: Brucellosis in Man and Animals, New York, Commonwealth Fund, 1943.

Doses of Drugs.—There was some variation in the amounts of drugs that were administered. In general, the dose of streptomycin was 1 gm. administered intramuscularly twice daily for seven to 14 days, with sulfadiazine given orally simultaneously in a dose of 1 gm. four times daily for 21 days. Patients treated with aureomycin alone were given 0.5 gm. orally four times a day for 10 to 21 days (average, 14 days). Those receiving only chloramphenicol were given 0.5 gm. four times a day for 10 days, or 0.25 gm. eight times a day for 10 days.

Follow-Up Period.—The average follow-up period for 18 of the 21 patients receiving streptomycin and sulfadiazine was 29.5 mo. Two of the patients treated with streptomycin and sulfadiazine subsequently received aureomycin, and insufficient information was available on a third. In the group treated with aureomycin the average follow-up period for 20 of the 22 patients was 15 mo. One patient was given chloramphenicol later, and adequate follow-up data were not available for another patient. The average follow-up period for the eight patients receiving chloramphenicol was 11 mo.

Evaluation of Results.—The patients were seen and examined periodically after receiving treatment. At the same time blood was obtained for agglutination tests and for cultures. The results are presented under four categories. The first category includes those patients who recovered promptly and who remained well without any residuals of the illness and without a relapse. These patients manifested a declining titer of agglutinins, and the blood cultures remained sterile. The second group includes those persons, with or without symptoms, who had positive blood cultures after treatment had been completed. The third group includes those persons who had temporary improvement following treatment but who suffered a relapse of symptoms. While the cultures for these patients remained sterile, a persistent elevation of the titer of Brucella agglutinins and the presence of fever and sweats provided objective evidence for the existence of active disease. The fourth group is comprised of those persons whose condition was improved but who complained of ill-health after treatment. Blood cultures remained sterile, and, as a rule, the titer of the agglutinins declined. There was little or no objective evidence of persistent disease. It was difficult to decide whether the symptoms of weakness, vague aches and pains, nervousness, and mental depression represented residuals of the infection, or whether they indicated the persistence of active disease. This aspect of brucellosis has been discussed in detail elsewhere.[7b]

RESULTS

Streptomycin and Sulfadiazine.—Data pertaining to the 21 patients who received streptomycin and sulfadiazine are presented in Table 1. Since the immediate results of treatment for some of these patients were previously published,[1h-j] the same case numbers used for the patients in the previous reports are employed in the present study. Eleven of the 21 treated patients recovered promptly and remained well without any residuals of the disease. Included in this group is one patient who had subacute bacterial endocarditis with involvement of the aortic valve due to Br. abortus (Patient 27). He still has a moderate amount of vertigo due to the streptomycin administered. A second patient with bacteremia and healed mitral stenosis also recovered completely (Patient 33). Another patient who recovered completely was a 32-yr.-old man (Patient 39) who had been ill for nine months with an excruciating pain involving the right sciatic nerve. Roentgenologic studies revealed a destructive process of the right sacroiliac joint.

There were six patients who had positive blood cultures after treatment was discontinued (34, 37, 44, 46, 47, 48). Two of these patients (46 and 47) received aureomycin subsequently. Observations on this group emphasize the importance of cultures after the completion of therapy, since two of the patients (44 and 48) appeared to have recovered completely without any complaints, although Br. abortus was recovered from their blood. It is significant that six of the 21 patients, or

28.5%, had positive blood cultures shortly after the conclusion of therapy. In no instance was the persistent bacteremia due to a streptomycin-resistant strain of Brucella.

Only one patient (30) who appeared to have recovered completely then suffered a late relapse. This patient had severe spondylitis with radiculitis. He was given a second course of streptomycin and sulfadiazine therapy, and, although the follow-up observations on him are incomplete, we have no knowledge of a relapse or of residuals of the disease. There were five patients who had some complaints of ill-health after therapy, although cultures were negative. One of the patients (35) had amebiasis and thrombophlebitis as complications, and the residuals could not be attributed to brucellosis. One of the most remarkable recoveries occurred in Patient 49, a 56-yr.-old farmwife, who had been ill for three years. She had been in bed for one year and in a tuberculosis sanatorium for several months, with a paravertebral abscess thought to be Pott's disease (tuberculosis of vertebra). This was proved to be spondylitis due to Br. abortus. Shortly after treatment had been discontinued, she was ambulatory. Except for some symptomatic residuals and limitation of motion of the back, she has remained well.

Aureomycin.—Of the 22 patients who were treated with aureomycin, 13 recovered completely without any relapse or residuals of the disease (Table 2). Two of these patients (46 and 47) had been treated previously with streptomycin and sulfadiazine, and one of these (Patient 46) died suddenly from a cerebral hemorrhage shortly after aureomycin therapy. This fatality did not appear to be related to the antecedent brucellosis. A remarkably rapid recovery in this group occurred in a farm boy (Patient 53) with suppurative arthritis of the hip joint. Br. abortus was recovered from the joint prior to treatment. Another rapid recovery occurred in a patient (70) who had a bacteremia due to Br. abortus and, in addition, an aortic regurgitation which was probably associated with a bicuspid aortic valve; he had had a previous surgical correction for coarctation of the aorta. The youngest patient ever seen and treated for brucellosis at the University Hospitals was a 1½-yr.-old boy (Patient 63), who had been ill a year. He recovered completely with treatment and has remained well. The chief manifestation of disease in this patient was severe and unexplained anemia.

A positive culture for Br. abortus was obtained from only one patient after therapy was completed. This was a 3½-yr.-old boy (Patient 55) who had arthritis of the hip. After a second course of treatment with aureomycin he recovered completely.

There were three patients who had a prompt recovery and appeared to be completely well but then underwent a relapse, though the blood cultures remained sterile. One of these patients (54) is of considerable interest because he apparently had recurrent infections, rather than relapses, prior to treatment with aureomycin. He was the father of Patient 55. He was a veterinarian specializing in obstetrics and was exposed repeatedly to Bang's disease (infectious abortion caused by Br. abortus) in infected herds of cattle.

There were five patients who had residual complaints of ill-health, although the blood cultures remained sterile and there was no other objective evidence of an active infection.

TABLE 1.—*Summary of Follow-Up Study on Twenty-One Patients Having Culturally*

No. of Patient	Age, Yr.	Sex	Occupation	Duration of Symptoms Prior to Therapy and Complications	Serologic and Bacteriologic Results Prior to Therapy	
					Titer of Agglutinins	Culture
27	45	M	Farmer	9 mo.; subacute bacterial endocarditis (aortic) due to Br. abortus	1:5,120	Br. abortus from blood 4×
28	28	F	Farmwife	9 mo.; radiculoneuritis, left arm	1:640	Br. abortus from blood 2×; bone marrow 1×
29	28	M	Farmer	2 mo.; mesenteric adenitis; appendicitis suspected initially	1:160	Br. abortus from blood 1×
30	32	M	Farmer	4 mo.; spondylitis and radiculitis	1:1,280	Br. abortus from blood 3×
31	57	M	Carpenter in meat-packing plant	3 wk.	1:320	Br. abortus from blood 1×
32	25	M	Laborer in meat-packing plant	2 mo.	1:1,280	Br. abortus from blood 2×
33	31	F	Clerk-typist in meat-packing plant	2 mo.; old mitral stenosis following attack of rheumatic fever at age 7 yr.	1:320	Br. abortus from blood 1×
34	21	M	Construction worker in meat-packing plant	3 wk.	1:5,120	Br. abortus from blood 3×
35	33	M	Highway engineer	1 mo.; amebiasis	1:5,120	Br. abortus from blood 2×
36	25	M	Laborer in meat-packing plant	1 wk.	1:1,280	Br. abortus from blood 2×
37	60	M	Farmer	3 mo.; radiculitis, right thorax	1:5,120	Br. abortus from blood 6×
38	31	M	Laborer in meat-packing plant	4 mo.	1:2,560	Br. abortus from blood 4×
39	32	M	Laborer in meat-packing plant	9 mo.; acute osteitis right sacroiliac joint with severe, incapacitating sciatic pain	1:2,560	Br. abortus from sternal bone marrow 1×; all blood cultures sterile
40	54	M	Farmer	4 mo.	1:1,280	Br. abortus from sternal bone marrow 1×; blood sterile
44	38	M	Brass polisher	2 wk.	1:2,560	Br. abortus from blood 4×
45	43	M	Millwright in meat-packing plant	2 mo.	1:5,120	Br. abortus from blood 1×
46	61	M	Farmer	6 mo.; hypertension; old cerebral thrombosis with left hemiplegia; chronic nephritis; recurring peptic ulcer	1:640	Br. abortus from blood 5×
47	47	M	Laborer in meat-packing plant	1 wk.	1:640	Br. abortus from blood 3×
48	30	M	Laborer in meat-packing plant	10 days; amebiasis	1:5,120	Br. abortus from blood 2×
49	56	F	Farmwife	3 yr.; spondylitis with paravertebral abscess; completely incapacitated 1 yr.	1:1,280	Br. abortus from blood 2×
50	20	F	Employee in meat-packing plant	6 wk.	1:2,560	Br. abortus from blood 1×

* Nine months after first course.
† Seventeen days after first course.
‡ Five days after second course.
§ Twenty days after third course.
‖ Intermittent doses were given for five months.

Proved Brucellosis Who Were Treated with Streptomycin and Sulfadiazine Concurrently

Drug Used	Dose, Gm.	Duration of Therapy, Days	Serologic and Bacteriologic Results After Therapy: Titer of Agglutinins	Culture	Immediate Results of Therapy and Final Outcome
Streptomycin	52	14	29 mo., 1:320	Sterile	Temperature normal in 12 days; aortic murmur persisted; no relapse; total follow-up, 39 mo.; residual vertigo from streptomycin
Sulfadiazine	120	22			
Streptomycin	57	15	1 wk., 1:320	Sterile	Temperature normal in 18 days; no relapse; residual fatigue but has worked since 1 mo. after therapy; total follow-up, 39 mo.
Sulfadiazine	120	22	5 mo., 1:160		
Streptomycin	22	8	27 mo., 0	Sterile	Complete recovery; no relapse; total follow-up, 37 mo.
Sulfadiazine	115	20			
First course:			9 mo. after first course, 1:320	Sterile blood 7×; sterile lymph node, spinal fluid, bone marrow	Prompt response to 1st course; relapse with low-grade fever and back pain 3 mo. later; good response to 2d course; no relapse as far as can be determined after 2d course
Streptomycin	14.5	8			
Sulfadiazine	142	24			
Second course*:					
Streptomycin	28	14			
Sulfadiazine	94	23			
Streptomycin	14	8	28 mo., 1:80	.....	Prompt decline of temperature in 5 days; complete recovery; total follow-up, 28 mo.
Sulfadiazine	124	21			
Streptomycin	14	7	3 mo., 1:320	Sterile	Prompt and complete recovery; total follow-up, 36 mo.
Sulfadiazine	76	12			
Streptomycin	14	7	24 mo., ±1:80	Sterile	Prompt and complete recovery; total follow-up, 33 mo.
Sulfadiazine	102	21			
First course:			16 days after 1st course, 1:1,280	Br. abortus from blood 4× after 1st course	Prompt drop in temperature and improvement after each course; relapse after 1st 3 courses—then complete recovery after final course; total follow-up, 30 mo. after last course
Streptomycin	16.5	9			
Sulfadiazine	66	9			
Second course†:			2 days after 3d course, 1:2,560	4× after 3d course	
Streptomycin	8	4			
Sulfadiazine	20	4			
Third course‡:			27 mo. after last course, 1:40	Sterile after final course	
Streptomycin	14	7			
Sulfadiazine	62	13			
Fourth course§					
Streptomycin	43.5	15			
Sulfadiazine	87	15			
Totals:					
Streptomycin	82	35			
Sulfadiazine	235	41			
Streptomycin	27	28	11 mo., 1:160	Blood sterile	Thrombophlebitis with multiple pulmonary infarcts developed followed by empyema with rib resection; no further illness attributable to brucellosis; total follow-up, 30 mo.
Sulfadiazine	181	25	12 mo., 1:80	18×	
Also antiamebic therapy			16 mo., 1:40		
Streptomycin	19	11	.....	Sterile 2× within 4 mo.	Prompt and complete recovery; total follow-up, 24 mo.
Sulfadiazine	74	13			
Sulfonamide alone	..	1 mo.	.....	Br. abortus from blood day after completion of therapy; subsequent culture sterile at end of 2 mo.	Slow recovery over period of 2 yr.; probably related to streptomycin toxicity; total follow-up, 30 mo.
Streptomycin	18	9			
Sulfadiazine	132	22			
Streptomycin	14.5	8	8 mo., 1:20	Sterile	Prompt and complete recovery; total follow-up, 24 mo.
Sulfadiazine	138	24			
Sulfonamide ‖	..	..	2 mo., 1:320	.....	Prompt improvement and complete recovery over 4½ mo. after therapy; no relapse; total follow-up, 30 mo.
Streptomycin	28.5	14	19 mo., 1:40		
Triple sulfonamides	130	35	30 mo., absent		
Streptomycin	30.8	15	6½ mo., 1:320	.....	Prompt improvement and complete recovery; total follow-up, 31 mo.
Sulfadiazine	44.8	15	31 mo., ±1:80		
Streptomycin	26	14	6 days, 1:2,560	Br. abortus from blood 6 days after therapy	Prompt improvement and complete recovery; total follow-up, 18 mo.
Sulfadiazine and sulfamerazine	180	21			
Streptomycin	28	14	16 mo., 1:320	Sterile	Prompt and complete recovery; total follow-up, 26 mo.
Sulfadiazine	186	21	19 mo., 1:320		
			26 mo., 1:160		
First course:			1 mo., 1:320	Br. abortus from blood 2×, 3 and 4 days after therapy	Improvement after therapy but low-grade fever, chills, backache and bacteremia persisted; see Table 2
Streptomycin	16	11			
Sulfadiazine	56	11			
Streptomycin	23	16	1 mo., 1:320	Blood: sterile 3× in 1 mo. after therapy; Br. abortus 2 mo. after therapy; then sterile 10× in 6 wk.	Remission of fever and symptoms; relapse with bacteremia and fever; see Table 2
Sulfadiazine	92	16	2 mo., 1:640		
Streptomycin	29	15	1 mo., 1:2,560	In 3 mo. after therapy Br. abortus from blood 4×, from bone marrow 1×; subsequent cultures sterile	Prompt recovery and felt well although bacteremia persisted for 3 mo. after therapy; no clinical relapse; total follow-up, 26 mo.; doing hard physical labor on section crew
Sulfadiazine	62	15	3 mo., 1:320		
			8 mo., 1:160		
			28 mo., 1:160		
Streptomycin	27.5	15			
Sulfadiazine	90	15			
.....	..	..	14 mo., ±1:160	Sterile	Striking improvement; relief of back pain, sweats; fusion of T_7 and T_8; residual of mild nervousness, depression, stiffness of back; no relapse; total follow-up, 24 mo.
Streptomycin	20	10	2 mo., 1:640	.....	Prompt and complete recovery; total follow-up, 26 mo.
Sulfadiazine	75	15			

TABLE 2.—*Summary of Follow-Up Study on Twenty-Two Patients with*

No. of Patient	Age, Yr.	Sex	Occupation	Duration of Symptoms Prior to Therapy and Complications	Serologic and Bacteriologic Results Prior to Therapy	
					Titer of Agglutinins	Culture
46	61	M	Farmer	See Table 1; because of persistence of symptoms and bacteremia treated with aureomycin and sulfadiazine	1:320	Br. abortus from blood 2× after streptomycin and sulfadiazine
47	47	M	Laborer in meat-packing plant	See Table 1; because of recurrence of symptoms treated with aureomycin	1:640	Br. abortus from blood 1× after streptomycin and sulfadiazine
51	17	M	Farm laborer	3 wk.	1:2,560	Br. abortus from blood 5×
53	12	M	Farm boy	13 mo.; brucella arthritis, left hip, with destruction of bone and flexion contracture	1:160	Br. abortus from blood 2×; from aspirated fluid from left hip joint
54	26	M	Veterinarian	8 mo.	1:640	Br. abortus from blood 1×
55	3½	M	Son of patient 54	6 mo.; arthritis of right hip	1:5,120	Br. abortus from blood 3×
56	56	F	Farmwife	3 mo.	1:2,560	Br. abortus from blood 3×
57	22	M	Laborer in meat-packing plant	7 wk.	1:1,280	Br. abortus from blood 2×
58	33	F	Farmwife	7 mo.	1:1,280	Br. abortus from blood 4×
59	19	M	Laborer in meat-packing plant	6 wk.; jaundice and albuminuria; liver biopsy specimen showed focal hepatitis with granulomas	1:1,280	Br. abortus from blood 2×
60	39	M	Farmer	2 wk.	1:160	Br. abortus from blood 2×
61	31	M	Laborer in meat-packing plant	10 days; amebiasis	1:640	Br. abortus from blood 5×, sternal bone marrow 1×
62	23	M	Laborer in meat-packing plant	13 mo.	1:640	Br. abortus from blood 2×
63	1½	M	.....	About 1 yr.; severe anemia	1:2,560	Br. abortus from blood 2×, from bone marrow 1×
64	52	M	Laborer; cleaning cattle trucks	3 wk.; positive serologic reaction for syphilis	1:5,120	Br. abortus from blood 1×, from sternal bone marrow 1×
65	32	M	Physician	1 wk.	1:640	Br. melitensis from blood 1×
66	35	M	Furniture buyer	3 wk.	1:640	Br. abortus from blood 3×, from bone marrow 1×
67	27	M	Farmer	2 wk.	1:160	Br. abortus from blood 3×
68	23	M	Farmer	4 mo.	1:320	Br. abortus from blood 1×
69	35	M	Radio mechanic	2½ mo.	1:1,280	Br. abortus from blood 1×
70	22	M	Student	2 mo.; aortic regurgitation; surgical correction of coarctation of aorta 15 mo. prior to onset of illness	1:1,280	Br. abortus from blood 1×
71	33	M	Carpenter in meat-packing plant	3 wk.	1:1,280	Br. abortus from blood 1×

* Patient was treated one year previously with penicillin and sulfadiazine. Relapse occurred after one year; then aureomycin therapy was begun.
† Patient first received irregular doses of penicillin, streptomycin, and Brucella vaccine.
‡ Five weeks after first course.
§ Four weeks after first course.
‖ One year after first course.
¶ Five months after first course.

Culturally Proved Brucellosis Who Were Treated with Aureomycin

Drug Used	Dose, Gm.	Duration of Therapy, Days	Serologic and Bacteriologic Results After Therapy: Titer of Agglutinins	Serologic and Bacteriologic Results After Therapy: Culture	Immediate Results of Therapy and Final Outcome
Aureomycin Sulfadiazine (concurrently)	28.5 79	16 16	1 mo., 1:640	Sterile	Continued improvement in strength and well-being 4 mo. after therapy; sudden cerebral hemorrhage, died in 6 wk.; autopsy revealed encephalomalacia, cerebral arteriosclerosis
Aureomycin	28	14	1 mo., 1:320 5 mo., 1:80 10 mo., 1:80 15 mo., 1:40	Sterile 8× in 6 mo.	No relapse; total follow-up, 21 mo.; had cholecystectomy for stones; liver biopsy showed portal cirrhosis and hemosiderosis; culture of bile, cystic lymph node, and liver negative for brucella
First course: Aureomycin Second course: Aureomycin	 24 51	 12 18	6 wk after 1st course, 1:320 25 mo. after 2d course, ±1:40	Sterile 7× after 1st course	Prompt recovery; clinical relapse 6 wk. after 1st course of therapy; prompt recovery after 2d course with no relapse; total follow-up, 25 mo.
Aureomycin*	28	14	21 mo., 1:80	.....	Prompt and complete recovery; no relapse; total follow-up, 21 mo.
Aureomycin†	37	16	17 mo., ±1:160	Sterile 6× in 18 mo.	Rapid improvement with residual of intermittent fatigue, headache for 1 yr.; then complete recovery; total follow-up, 15 mo.
First course: Aureomycin Second course‡: Aureomycin	 8.6 10	 14 10	5 wk. after 1st course, 1:640 4 mo., 1:320 15 mo., ±1:80	Br. abortus from blood 2× after 1st course within 5 wk.; sterile after 2d course 2× in 15 mo.	Rapid improvement with disappearance of pain in right hip after 1st course; slight recurrence of pain, right hip; fever and bacteremia persisted; complete recovery after 2d course; no relapse; total follow-up, 15 mo.
Aureomycin	32	14	6 mo., 1:640 17 mo., 1:160	Sterile	Prompt improvement; residual fatigue for 18 mo.; no relapse; total follow-up, 17 mo.
Aureomycin	22	11	2 wk., 1:320 16 mo., 1:160	Sterile 3×	Prompt and complete recovery; total follow-up, 16 mo.
Aureomycin	26	14	1 mo., 1:1,280 3 mo., 1:640 16 mo., 1:40	Sterile 3× in 3 mo.	Prompt improvement with residual fatigue, headaches for 1 yr.; then complete recovery; total follow-up, 15 mo.
Aureomycin	16	19	1 mo., 1:320 2 mo., 1:160 6 mo., ±1:80 10 mo., ±1:80 16 mo., ±1:20	Sterile 5× in 1 mo.	Prompt and complete recovery; no relapse; total follow-up, 16 mo.
Aureomycin	20	10	5 wk., 1:320	Blood sterile 6×; sternal bone marrow sterile 2×	Prompt and complete recovery; no relapse; total follow-up, 16 mo.
First course: Aureomycin Second course§: Aureomycin	 20 20	 10 10	5 wk. after 1st course, 1:320 4 wk. after 2d course, 1:320 10 mo., 1:80 18 mo., ±1:160	Blood culture sterile 15× in 2 mo.	Prompt improvement; clinical relapse in 1 mo.; improvement after 2d course; chronic fatigue persisted though cultures sterile and agglutinins had declined; re-treated with terramycin and then chloramphenicol; presence of active disease doubtful; total follow-up, 18 mo.
First course: Aureomycin Second course‖: Aureomycin	 20 27	 10 27	11 mo. after 1st course, 1:160 16 mo. after 1st course (5 mo. after 2d course), 1:80	Blood culture sterile 7× in 1 yr.	Prompt improvement; residual of chronic fatigue; no improvement from 2d course of therapy; doubtful if active disease present with sterile cultures and declining agglutinin titer; total follow-up, 16 mo.
Aureomycin	14	16	.....	Blood sterile 3× in 15 mo.	Prompt and complete recovery; no residual; total follow-up, 15 mo.
Aureomycin	29	15	16 mo., 1:320	Blood sterile	Prompt and complete recovery; no residual; total follow-up, 16 mo.
Aureomycin	40	20	5 mo., ±1:320 15 mo., ±1:80	Blood sterile	Prompt and complete recovery; no relapse; total follow-up, 15 mo.
Aureomycin	22	11	1 mo., 1:640 15 mo., 1:40	Blood sterile 3×	Prompt and complete recovery; no relapse; total follow-up, 15 mo.
Aureomycin	20	10	3 mo., 1:80 4 mo., 1:80 11 mo., ±1:40	Blood sterile 9× in 3 mo.	Prompt improvement; well for 2 mo.; then recurrence of sweats, fever, backache; cultures persistently negative with drop in agglutinin titer; marked psychoneurosis; treated with chloramphenicol later; see Table 3
Aureomycin	20	10	.....	Blood sterile 3× in 5 wk.	Prompt and complete recovery; no relapse as far as known in 12 mo.; total follow-up, 12 mo.
Aureomycin	31	16	1 mo., 1:640	Blood sterile 2× in 6 wk.	Prompt and complete recovery; no relapse; total follow-up, 11 mo.
Aureomycin	28	14	2 mo., 1:640 5 mo., 1:640 9 mo., 1:640	Blood sterile 4× in 9 mo.	Prompt and complete recovery; no relapse; total follow-up, 9 mo.
First course: Aureomycin Second course¶: Aureomycin	 20 15	 10 10	5 mo. after 1st course, 1:2,560 12 mo., 1:2,560	Blood sterile 2× after 1st course of therapy, 2× after 2d course	Prompt recovery with no symptoms until 4 mo. after therapy; then fever, headache, sweats; cultures sterile; after 2d course recovery with no relapse; total follow-up after 2d course of therapy, 7 mo.; of relapse or reinfection

TABLE 3.—*Summary of Follow-Up Study on Eight Patients with*

No. of Patient	Age, Yr.	Sex	Occupation	Duration of Symptoms Prior to Therapy and Complications	Serologic and Bacteriologic Results Prior to Therapy	
					Titer of Agglutinins	Culture
67	27	M	Farmer	See Table 2; given chloramphenicol because of recurrence of symptoms of night sweats and backache 2 mo. after treatment with aureomycin	±1:160	9 blood cultures sterile in 3 mo. after aureomycin therapy
72	29	M	Elevator operator in meat-packing plant	2 wk.; medical discharge from military service 5 yr. prior to present illness because of psychoneurosis	1:1,280	Br. abortus from blood 3×
73	17	M	Farmer	1 mo.	1:1,280	Br. abortus from blood 1×
74	29	M	Laborer in meat-packing plant	3 wk.	1:640	Br. suis from blood 5×
75	26	M	Laborer in meat-packing plant	1 mo.; psychopath; discharged from military service because of "incompetence"	1:1,280	Br. suis from blood 5×
76	22	M	Hardware dealer	1 mo.	1:1,280	Br. abortus from blood 2×, from bone marrow 1×
77	24	M	Veterinarian	1 wk.	1:640	Br. abortus from blood 1×
78	26	M	Laborer in meat-packing plant	1 mo.	1:10,240	Br. melitensis from blood 2×

Chloramphenicol.—Only eight patients were treated with chloramphenicol (Table 3). One of these persons (Patient 67) had been treated previously with aureomycin. Chloramphenicol therapy was without effect on this patient's illness. It was the consensus that he had a definite psychoneurosis, though active Brucella infection may also have been present. Prompt and complete recovery occurred in two patients without any subsequent relapses or residuals of the infection. One patient (72) had persistently positive blood cultures after the completion of therapy. He continued to have residual complaints after a subsequent course of therapy with dihydrostreptomycin and aureomycin and then with aureomycin alone. While no relapses were encountered, there were four patients with residual complaints after therapy had been discontinued.

COMMENT

This investigation of the results of therapy in bacteriologically proved cases of brucellosis demonstrates quite clearly the remarkable advances that have taken place in the treatment of this disease. The effectiveness of treatment with streptomycin and sulfadiazine, aureomycin, or chloramphenicol has been demonstrated in four ways. First, of the 48 patients treated, the condition of all but one (67) was improved after therapy. Within 48 to 72 hr. after the initiation of treatment, there was a drop in the temperature, the toxemia abated, and pain was allayed. In 26 of the patients, this improvement was prompt and was succeeded by complete recovery. Second, serious and painful complications were abruptly controlled, and

Culturally Proved Brucellosis Who Were Treated with Chloramphenicol

Drug Used	Dose, Gm.	Duration of Therapy, Days	Serologic and Bacteriologic Results After Therapy: Titer of Agglutinins	Serologic and Bacteriologic Results After Therapy: Culture	Immediate Results of Therapy and Final Outcome
Chloramphenicol	22	10	1 mo., 1:80 8 mo., ±1:40	Blood culture sterile 2× in 8 mo.	No improvement; residual of backache, fatigue; definite psychoneurosis present; total follow-up, 7 mo.
Chloramphenicol	20	10	1 mo., 1:5,120 5 mo., 1:640	Br. abortus from blood 4× 3 to 8 wk. after therapy	Prompt improvement, but weakness and fatigue persisted with positive blood cultures; treated with aureomycin and dihydrostreptomycin; symptoms persisted; given course of aureomycin; blood cultures sterile; total follow-up, 5 mo.
Chloramphenicol	20	10	13 mo., 1:100	Blood sterile 6× in 1 mo.	Prompt improvement and complete recovery; no relapse; total follow-up, 13 mo.
Chloramphenicol	24	11	.....	Blood sterile at conclusion of therapy	Prompt improvement; returned to work and working regularly but has residual complaints of headaches, poor appetite, fatigue 15 mo. later; total follow-up, 15 mo.
Chloramphenicol	24	11	16 mo., 1:1,280	Blood sterile in following 16 mo.	Only temporary improvement; continued symptoms of headache, cough, fatigue with psychopathic personality; total follow-up, 16 mo.
Chloramphenicol	20	10	2 mo., 1:640	Immediately after treatment blood sterile 3×; 1 mo. after Br. abortus from blood	Prompt improvement; working steadily; slight fatigue; no further chemotherapy; total follow-up, 12 mo.
Chloramphenicol	22	11	2 mo., 1:1,280	Blood sterile 3×, immediately after therapy and 2 mo. after	Prompt and complete recovery; no relapse; total follow-up, 11 mo.
Chloramphenicol	24	23	1 mo., 1:5,120 2 mo., 1:2,560 4 mo., 1:1,280 8 mo., 1:320 9 mo., 1:320	Br. melitensis from blood 1 mo. after; 9 cultures sterile in succeeding 7 mo.	Prompt improvement; but headaches, weakness persisted; treated subsequently with 2 courses of aureomycin; complete recovery; 9 blood cultures sterile over 7 mo.; total follow-up, 10 mo.

progression of the lesions was prevented. These complications included hitherto fatal subacute bacterial endocarditis.[10] Painful and destructive bone lesions, such as spondylitis and suppurative arthritis, were also eliminated. In patients with organic valvular heart disease bacteremias due to Br. abortus were terminated, thus preventing the possible onset of Brucella endocarditis. Third, in no instance did a localizing complication occur after treatment was established. Fourth, the bacteremia in a majority of the patients was controlled.

It must be emphasized that we were primarily concerned with the treatment of brucellosis due to Br. abortus, since 44 of the 48 cases were due to this species. Since Br. melitensis and Br. suis cause severer illness than does Br. abortus, it will be highly desirable to have a comparable follow-up study of therapy in brucellosis caused by these more invasive strains. We have already demonstrated the immediate improvement that occurs in patients ill with Br. melitensis and treated with aureomycin.[1k]

The results of therapy did not appear to be influenced by the duration of illness prior to treatment. Patients who had been ill for several months seemed to respond as well as those ill for only a few weeks. Only four patients ill for one year or more were treated.

10. Spink, W. W., and Nelson, A. A.: Brucella Endocarditis, Ann. Int. Med. **13**:721, 1939. Spink, W. W.; Titrud, L. A., and Kabler, P.: A Case of Brucella Endocarditis with Clinical, Bacteriologic and Pathologic Findings, Am. J. M. Sc. **203**:797, 1942.

In a comparative evaluation of drugs used in the present study, there was no question that aureomycin was superior to the streptomycin-sulfadiazine combination. This superiority had been demonstrated previously in the studies carried out in México, D. F.[11] In addition to its ease of administration, aureomycin also had the advantage of causing no serious side effects. Two of the patients treated with streptomycin had a persistent, serious vestibular dysfunction. There were six patients who had bacteremia after completion of therapy with streptomycin and sulfadiazine, as compared with one after aureomycin treatment. However, in a subsequent group of patients treated with aureomycin but not followed long enough to include in this report, bacteremia has not been eliminated in three patients after two and three courses of aureomycin therapy. It is of interest that some of the latter patients had no fever and no complaints, although cultures of blood yielded Brucella after treatment with aureomycin. This re-emphasizes the observation that in the clinical investigation of the results of therapy in brucellosis multiple cultures of blood must be made after the completion of therapy, and preferably over a period of several weeks. The over-all results with aureomycin therapy were better than those obtained with use of chloramphenicol. These clinical results coincide with those observed in experimentally infected animals; it has been shown that on a weight-for-weight basis aureomycin offers more protection against brucellosis than chloramphenicol.[11]

There were also certain shortcomings in the treatments that were employed. In eight patients bacteremia persisted after the completion of treatment, six of the cases occurring in patients treated with the combination of streptomycin and sulfadiazine. In no instance was the persistent bacteremia associated with the appearance on cultures of Brucella resistant in vitro to the antibiotics employed in treatment, although previous studies in this clinic demonstrated that a highly resistant strain of Br. abortus could and did appear in a patient during treatment with streptomycin alone.[11a] It is not unlikely that the failure to eliminate the Brucella was related to foci of organisms not reached by the drugs. Brucella organisms tend to localize and remain viable within cells, and recent in vitro studies by Magoffin and Spink [12] have shown that intracellular Brucella organisms are protected against the lethal action of the antibiotics evaluated in the present investigation. In addition to the patients having positive blood cultures after treatment, there were five persons who had a clinical relapse without a bacteremia being demonstrated. All these patients received aureomycin. There were 14 patients who had residual complaints of ill-health after treatment had been completed. The symptoms of one of these patients were attributed to the streptomycin administered. In the remaining 13 patients the complaints included weakness, vague aches and pains, and nervousness. One of the most difficult problems in brucellosis is the proper evaluation of these subjective complaints. Objective data are often lacking to indicate the presence of active infection. The blood cultures remain sterile, and the titer of agglutinins declines. No fever is present, and the physical examination elicits few or no

11. Footnote deleted.

12. Magoffin, R. L., and Spink, W. W.: The Protection of Intracellular Brucella Against Streptomycin Alone and in Combination with Other Antibiotics, J. Lab. & Clin. Med. 37:924, 1951.

abnormalities. These subjective manifestations were quite prominent in another group of patients who received no antibiotic therapy and who were followed over a long period.[7a] The complaints appear to be a feature of the natural course of the disease. Such patients do not respond favorably to continued therapy with the antibiotics.

While it is apparent from these studies that considerable progress has been made in the therapy of brucellosis, further investigations with other drugs or combinations of drugs are necessary. One of the more recent developments is a modified therapy based upon the observations of Heilman,[13] who reported that a combination of dihydrostreptomycin and aureomycin was highly effective in controlling experimental brucellosis in mice. These observations have been confirmed by Werner and Knight [14] and by Magoffin [15] in our laboratory. Herrell and Barber [16] treated a group of patients with this combination and reported that of 35 patients with the culturally proved disease, 34 recovered completely after one course of therapy. In view of these findings, this combination is now being evaluated in our clinic. Herrell and Barber [16] recommended for the acutely ill patients that aureomycin be administered orally in a dose of 750 mg. every six hours for 12 to 14 days, and that 1 gm. of dihydrostreptomycin be given intramuscularly twice daily for the same period. In patients with localizing lesions, such as spondylitis, the same dose of aureomycin is to be given for 28 days, and 0.5 gm. of dihydrostreptomycin is to be administered intramuscularly twice daily for the same period. They state that no toxic reactions from dihydrostreptomycin were observed. However, delayed hearing loss has been attributed to use of this antibiotic.[17]

SUMMARY

A follow-up study was made on 48 patients with bacteriologically proved brucellosis who were treated with one of the following: a combination of streptomycin and sulfadiazine, aureomycin, or chloramphenicol. Brucella abortus was the cause of illness in 44 of the 48 patients.

Among 21 patients treated with streptomycin and sulfadiazine, there were 11 persons who promptly recovered and remained well; six patients had positive blood cultures after treatment, although two of these persons had no complaint of ill-health, and five others had residual complaints of ill-health.

Among 22 patients receiving aureomycin, 14 recovered promptly without a relapse; 3 patients had a relapse, 1 with a positive blood culture, and 5 patients had

13. Heilman, F. R.: The Effect of Combined Treatment with Aureomycin and Dihydrostreptomycin in Mice, Proc. Staff Meet., Mayo Clin. **24**:133, 1949.

14. Werner, C. A., and Knight, V.: The Suppressive Effect of Antimicrobial Drugs on Brucella Melitensis Infection in Mice, J. Immunol. **65**:509, 1950.

15. Magoffin, R. L.: Unpublished observations.

16. Herrell, W. E., and Barber, T. E.: A New Method for Treatment of Brucellosis, J. A. M. A. **144**:519 (Oct. 14) 1950.

17. Allison, S. T.; Volk, R., and Vitagliano, G. R.: Dihydrostreptomycin in the Treatment of Pulmonary Tuberculosis with Particular Reference to Its Toxicity as Compared with That of Streptomycin, New England J. Med. **241**:52, 1949. Shane, S. J., and Laurie, J. H.: Neurotoxicity of Dihydrostreptomycin, Canad. M. A. J. **63**:277, 1950.

residuals of ill-health. Of eight patients receiving chloramphenicol, two recovered completely; one showed no improvement; the bacteremia persisted in one, and four had residuals of ill-health.

This series included several patients with severe complications, such as spondylitis, suppurative arthritis, radiculitis, and subacute bacterial endocarditis. In every instance these complications were brought under control as a result of treatment. While this indicates quite clearly a definite advancement in the therapy of brucellosis, a significant number of patients relapsed or had residual complaints of ill-health. Further therapeutic investigations should be made with the combination of dihydrostreptomycin and aureomycin.

CHANGES IN SERUM COMPLEMENT DURING THE COURSE AND TREATMENT OF GLOMERULONEPHRITIS

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THE SUGGESTION that glomerulonephritis, as seen clinically, is a manifestation of an antigen-antibody reaction was first made by Schick [1] in 1907. This concept found some acceptance, although it was based at that time mostly on circumstantial evidence. Volhard [2] also emphasized and endeavored to prove this theory. Additional support was given the concept when Masugi [3] succeeded in producing, experimentally, a clinical and morphologic picture identical with human glomerulonephritis by injecting heterologous nephrotoxic serum. Masugi believed that the glomerulonephritis thus produced in animals was caused directly by the union of the injected antibody with the glomerular tissue, which acted as a fixed antigen. Kay,[4] however, has suggested that a more complicated system is involved in the pathogenesis of nephrotoxic nephritis, since the signs of nephritis do not appear immediately upon injection of the nephrotoxic serum but only after an interval of several days. The experiments of Masugi and Kay have given great impetus to the study of the immunologic mechanism of nephritis.

Sarre and Wirtz [5] demonstrated that specific selective absorption of nephrotoxic serum occurs in the kidney and believed that this initiates the immunologic mechan-

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From the Department of Medicine (Dr. Lange, Dr. Graig, and Dr. Oberman) and the Department of Pediatrics (Dr. Slobody and Dr. Ogur) of the New York Medical College and the New York Medical College Research Unit (Metropolitan Hospital).

1. Schick, B.: Die Nachkrankheiten des Scharlach, Jahrb. Kinderh. **65**:132, 1907.

2. Volhard, F., and Fahr, T.: Die Brightsche Nierenkrankheit: Klinik, Pathologie und Atlas, Berlin, Julius Springer, 1914.

3. Masugi, M.: Über die experimentelle Glomerulonephritis durch das spezifische Antinierenserum, Beitr. path. Anat. **92**:429, 1934.

4. Kay, C. T.: The Mechanism of a Form of Glomerulonephritis: Nephrotoxic Nephritis in Rabbits, Am. J. M. Sc. **204**:483, 1942.

5. Sarre, H., and Wirtz, H.: Geschwindigkeit und Ort der "Nephrotoxin"-Bindung bei der experimentellen Glomerulonephritis, Klin. Wchnschr. **18**:1548, 1939.

ism. Pressman,[6] by means of radioactive tracers, recently demonstrated the direct selective absorption of nephrotoxic serum by the glomerular apparatus.

Cavelti [7] produced a picture similar to human glomerulonephritis in a high percentage of rabbits by injecting homologous kidney extracts which had been incubated with hemolytic streptococci. Schwentker and Comploier,[8] in addition, demonstrated, with the use of complement-fixation tests, the presence of antibodies to kidney in a majority of patients with scarlet fever within one to two weeks after the acute phase. Antibodies resembling these were found in only a small number of normal persons. Kay and associates,[9] however, could not confirm the presence of such antibodies in similar cases.

In our studies with the fluorescein technique on the pathogenesis of edema formation, we found that the edema of acute glomerulonephritis and especially that of the nephrotic phase was, in a large part, due to increased capillary permeability.[10] In search for a possible "allergic" factor as a cause for such diffuse damage to the capillary network, the immunologic basis of glomerulonephritis was investigated. We showed,[11] by means of a modified collodion-particle technique,[12] that in 78% of cases of active glomerulonephritis antibodies to human kidney can be demonstrated in the serum. The appearance of free antibodies is less frequent during the early phases of the disease. Their presence is of great significance for the understanding of the character of the disease as an antigen-antibody reaction and can also be used for differential diagnostic and therapeutic purposes. These findings were recently confirmed by Pfeiffer and Bruch.[12a]

Attempts were made by us to modify and simplify the highly sensitive but, therefore, somewhat unreliable collodion technique in order to permit its use as a routine clinical procedure. In spite of extensive studies these attempts failed. We then resorted to the use of the complement-fixation test as applied in the Wassermann and Kolmer techniques. As previously described,[11a] aqueous infant-kidney extracts were used as antigen.

With the routine technique no positive results were obtained, but antibodies were shown to be present if the test's sensitivity was increased by the use of border-

6. Pressman, D.: The Zone of Localization of Antibodies, J. Immunol. **63**:375, 1949.

7. Cavelti, P. A., and Cavelti, E. S.: Studies on the Pathogenesis of Glomerulonephritis: I. Production of Autoantibodies to Kidney in Experimental Animals, Arch. Path. **39**:148 (March) 1945.

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11. (*a*) Lange, K.; Gold, M. M. A.; Weiner, D., and Simon, V.: Autoantibodies in Human Glomerulonephritis, J. Clin. Invest. **28**:50, 1949. (*b*) Lange, K.; Weiner, D.; Gold, M. M. A.; Tchertkoff, V., and Simon, V.: Autoantibodies in Different Phases of Human Glomerulonephritis, Bull. New York Acad. Med. **25**:447, 1949.

12. Cannon, P. R., and Marshall, C. E.: An Improved Serologic Method for the Determination of Precipitative Titers of Antisera, J. Immunol. **38**:365, 1940.

12a. Pfeiffer, E. F., and Bruch, H.: Autoantikörper gegen menschliches Nierengewebe, Verhandl. deutsch. Gesellsch. inn. Med., Kong. 56, p. 189, 1950. Pfeiffer, E. F.: Über zeitliche und quantitative Beziehungen zwischen Herd und Organleiden, Ztschr. Rheumaforsch. **10**:177, 1951.

line amounts of complement (C′) and hemolysin. This modified technique indicated that we were dealing with a complement-binding reaction, in which the small amounts of free antibodies present in the blood bound only minute amounts of complement when they reacted with kidney antigen.

When it became evident that the antigen-antibody reaction in glomerulonephritis was complement-binding, a study of complement titers in serums of patients during the various phases of glomerulonephritis was initiated. Gunn,[13] in a study of complement in exanthems, found that in four cases of postscarlatinal nephritis complement was low. Veil and Buchholz,[14] in their studies on complement in rheumatic fever, mention the low titers which they obtained in a number of cases of acute glomerulonephritis. Kellett and Thomson [15] examined 38 patients with nephritis for complement content. Despite their use of a relatively insensitive method, they found that in every patient with acute glomerulonephritis whom they examined within four weeks of onset the complement titers were much lower than normal. Their group did not include any patient in the nephrotic phase or any patient with chronic progressive active glomerulonephritis. A group of 12 patients in uremia or preuremia, which in several was a result of inactive glomerulonephritis as shown at postmortem study, did not exhibit a decreased complement titer. Reader [16] studied 16 patients with acute nephritis for complement content, using complete hemolysis as the point of reading. Despite this insensitive method, all patients showed lowered complement during the acute phase.

In our studies an attempt was made to use a well-standardized method which would be highly sensitive, would permit the use of small amounts of blood, could be repeated frequently, and would, thus, be applicable even to cases of acute nephritis in small children.

METHOD

The method used, explained in Table 1, follows the technique outlined by Wadsworth.[17] It cannot be stressed strongly enough that in order to obtain a high degree of sensitivity reading at 50% hemolysis is imperative. We compared the results obtained with reading done at 50% hemolysis with those at complete hemolysis. It was found that in a high percentage of tests there were no or only minor deviations from the normal range with readings done at 100% hemolysis. Definite deviations from normal were demonstrable at 50% hemolysis.

The Hemolytic System.—Fresh sheep cells were used between the second and eighth day after bleeding of the animals. The cells were kept in a 3% sodium citrate solution at a temperature of approximately 5 C. and washed in isotonic sodium chloride solution at least three times until the supernate was crystal clear. A 5% sheep-cell solution in 0.9% saline solution was prepared from the washed and packed sheep cells immediately before use. The solution was standardized in a photoelectric colorimeter for constant hemoglobin content. Three hemolytic units of a commercial sheep-cell hemolysin (Lederle) was used in the amounts shown in Table 1.

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13. Gunn, W. C.: The Variation in the Amount of Complement in the Blood in Some Acute Infectious Diseases and Its Relation to the Clinical Features, J. Path. & Bact. **19**:155, 1914.

14. Veil, W. H., and Buchholz, B.: Der Komplementschwund im Blute, Klin. Wchnschr. **11**:2019, 1932.

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17. Wadsworth, A. B.: Standard Methods of the Division of Laboratories and Research of the New York State Department of Health, ed. 2, Baltimore, Williams & Wilkins Company, 1939.

The Serums.—Serums were used within 24 hours of collection. Immediately upon receipt, the serum was separated from the red cells after centrifugation and kept in a refrigerator at 5 C. until use.

Complement Unit.—Freshly prepared guinea-pig complement from commercial lyophilized guinea-pig serum was used for standardization of the system. Dilutions in steps of 1:5 of the complement from 1:5 to 1:50 were prepared, and to 0.1 cc. of the complement thus diluted, 0.1 cc. of an inactive serum, 0.2 cc. of saline solution, and 0.2 cc. of sensitized cells were added. It was found that 50% hemolysis was observed when standard guinea-pig complement was in 1:25 dilution. The titration was read by comparing the tubes with a color row consisting of tubes with decrements of 5% hemolysis, as described by Wadsworth.[17] The dilution of freshly prepared guinea-pig complement which just produces 50% hemolysis was called 1 complement unit. This was almost uniformly a dilution of 1:25.

The Test.—The tests were set up in the way described in Table 1. After an initial incubation of the serum dilutions for 90 min., the hemolytic system was added, and the tubes were incubated for 60 min. in a water bath of 37 C. The initial incubation for 90 min. was introduced only in order not to deviate from the procedure used when the same serums were tested for the presence of complement-binding antibodies. The tubes were kept for 24 hr. in a refrigerator at 5 C. Reading was done by selecting the tube which showed 50% hemolysis when matched with the 50%-hemolysis tube of the color-standard row. The dilution of the individual serum under

TABLE 1.—*Technique of Complement Titration of Active Serum*

Active serum dilution, 0.2 cc.	Straight	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Saline solution, cc.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Incubated in water bath for 90 min. at 37 C.								
Hemolysin, cc. (3 units of hemolysin)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
5% sheep-cell solution, cc.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Incubated in water bath for 60 min. at 37 C.								
24 hr. in refrigerator at +5 C.								
Reading for 50% hemolysis								

investigation which just produced 50% hemolysis was noted, and the complement content in units was calculated by dividing the dilution of the test serum by the dilution of the standard complement needed to produce 50% hemolysis. If none of the tubes of the test matched exactly the 50% hemolysis of the standard, the tubes closest to 50% (above and below) were evaluated for percentage of hemolysis, and the serum dilution corresponding exactly to 50% was determined by interpolation. With this method the results can be reproduced within close limits. The use of a colorimeter to determine the percentage of hemolysis was omitted since lipemic serums, because of their opalescence, tended to give false values in low dilutions.

RESULTS

Controls.—Seventy-seven determinations for serum complement content were made on 68 normal subjects or patients admitted with unrelated diseases. Fifty-four of our patients were adults, while 14 were children below the age of 12 years. The values obtained varied between 1.0 and 3.0 units, with an average of 1.68 units. It is well known that serum complement of normal persons is fairly constant.[18] We examined the blood of the same subject six times over a period of four months and found a variation of not more than ± 6%. The percentage distribution of the serum complement of the normal persons and those with unrelated diseases is shown in Figure 1. In this group were included five patients with high fever due to broncho-

18. Lange, C.: Complement Fixation and Blood Chemistry in Icterus, Uremia and Hydremia. Klin. Wchnschr. **1**:1040, 1922.

pneumonia or lobar pneumonia, and six febrile patients with acute tonsillitis. Patients with generalized eczema, inactive rheumatic fever, and urticaria due to penicillin are included in the group. These patients did not have any deviations of serum complement as compared with healthy normal controls. Three patients with advanced cirrhosis of the liver, not included among the controls, all had a moderate decrease of complement titers. This had been described previously by Goldner.[19] One patient with severe bilateral cavernous pulmonary tuberculosis was examined and found to have a very low complement (0.2 unit). The patient died two days later. It is well known that complement drops in the terminal stage of many diseases.

Acute Glomerulonephritis.—Forty-one determinations were made for 14 patients with acute glomerulonephritis whose onset varied from two weeks to 20 months

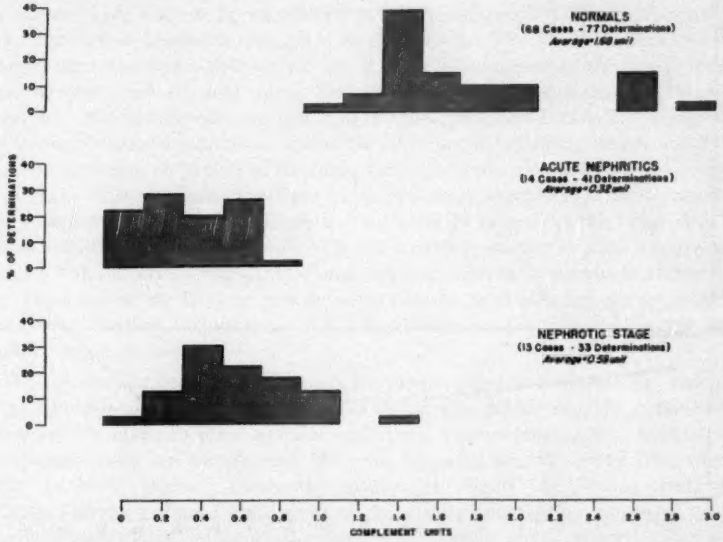

Fig. 1.—The percentage distribution of serum complement in controls in patients with acute nephritis, and in patients in the nephrotic stage.

prior to the first examination. Of this group five were children under 12 years of age. The criteria used for the diagnosis of glomerulonephritis were hematuria and albuminuria of varying degree, the presence of casts in the urine, normal serum cholesterol, frequently the presence or history of edema, especially facial, varying degrees of recently occurring hypertension, and, in most cases, a history of infection of the upper respiratory tract or other infection one to two weeks prior to the onset of the symptoms. All patients thus selected had a marked decrease of complement, the values ranging between 0 and 0.8 unit, with an average of 0.32 unit. The percentage distribution of the results obtained is shown in Figure 1. Two patients whose conditions were initially diagnosed as acute glomerulonephritis clinically had per-

19. Goldner, M.: Untersuchungen über das Komplement im Serum bei Leberkranken, Deutsche med. Wchnschr. **55**:390, 1929.

sistently normal complement titers. Both turned out to be urologic problems; one patient had a ureteral stone and the other pyelonephritis due to Proteus vulgaris (Bacillus proteus).

When healing occurs in glomerulonephritis, the complement returns to normal (Fig. 2). The complement content is usually still slightly depressed when all clinical and laboratory signs subside. Frequently, the normal range is reached within one week after complete clinical recovery. Whether the Addis count is elevated during the period in which complement level is still slightly depressed and all other clinical signs have subsided is at present under investigation. It is probable, however, that

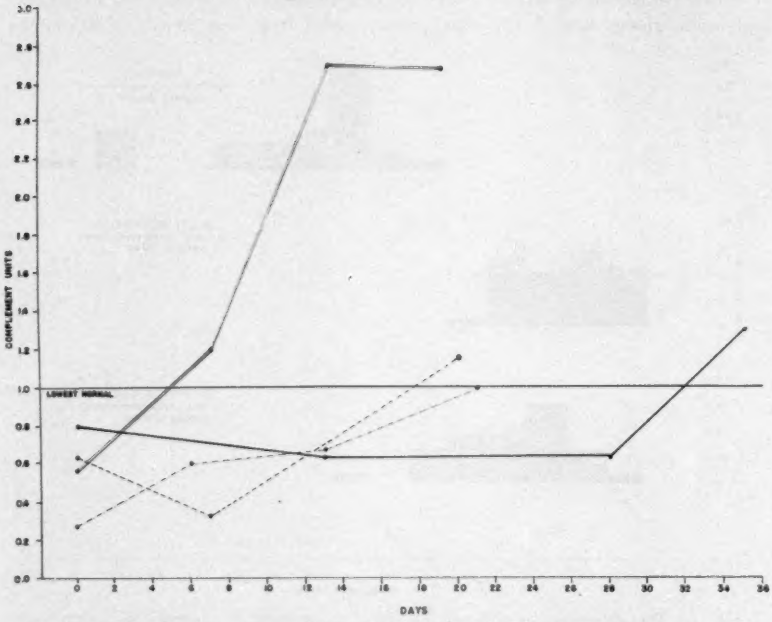

Fig. 2.—Behavior of complement during the healing of acute nephritis. Levels for various patients are indicated as follows: double line, N.M.; broken line, F.F.; line of dots and dashes, I.L., and solid line, D.M.

this slightly prolonged depression of complement content is due to the time required by the body to replenish it.

We are currently following eight patients whose complement content is persistently low and who also have had clinical signs of activity for two years or more. The statement [16] that complement is depressed only during the first two weeks of acute glomerulonephritis is, therefore, not valid when sufficiently sensitive methods are used in determinations of complement titers.

Two patients with acute nephritis had a complete absence of complement. The condition of one went downhill rapidly, and she died in uremia. The other is doing fairly well clinically, but moderate amounts of red blood cells, casts, and albumin in the urine and a high antistreptolysin titer (1,400) persist.

The Nephrotic Stage.—Thirty-three determinations were made in 13 cases clinically diagnosed as "nephrosis." Five of these cases were of children under 12 years of age. The values obtained ranged between 0.09 and 1.3 unit, with an average of 0.59 unit. The percentage distribution of the values is shown in Figure 1. The clinical diagnosis of this condition was based on the presence of marked generalized edema, proteinuria (between 10 and 30 gm. per day), elevated blood cholesterol, low blood albumin, and low osmotic pressure of the serum. All the patients of this group, with the exception of two, showed low complement values persistently. Although, in general the values are not quite as low as those found in patients with acute glomerulonephritis, they are definitely below the normal values.

The two patients who showed normal complement are of interest. One was a child with a complement titer of 1.0 unit, just at the lower normal range, who could be examined only once as he was discharged from the hospital shortly thereafter, before any further detailed studies could be carried out. The other case was that of a Negro man who had a definite history of acute glomerulonephritis nine months preceding the onset of mild ankle edema. The man, in addition, had severe alcoholism. He had hypoproteinemia, and the albumin-globulin ratio was inverted. The serum cholesterol was elevated, and the cephalin-flocculation reaction was 2+. His daily excretion of protein in the urine varied between 15 and 29 gm. Despite the presence of liver damage and signs of the nephrotic stage, both of which should lower complement, this patient had normal complement values. A third case with a normal complement content not entered in the statistics was that of a child who was admitted with moderate generalized edema and had received 16 plasma transfusions prior to admission, the last one just three days before determination of the complement titers. Further examinations of this child were not possible, since it left the hospital a few days later.

The complement content was low in all nephrotic patients who clinically and by history had no evidence of a preceding acute glomerulonephritis and who at the time of examination failed to show signs of underlying glomerulonephritis. Several of these patients with low complement, however, displayed uremia shortly thereafter, baring the basic disease. There was no trend to higher complement levels in nephrotic patients without clinical signs of glomerulonephritis when compared with those with such signs. It appears, therefore, at present highly doubtful whether there is a pure "nephrosis" considered from the immunologic point of view. It seems that in most, if not all, cases of the nephrotic stage the immunologic mechanism is identical with that of active glomerulonephritis.

Uremia.—We examined six patients in uremia, all with a distinct history of preceding glomerulonephritis. Two of these patients had uremia directly following acute glomerulonephritis, and both had low complement values (0.0 to 0.4 unit). Both had a rapid progression of the uremia with resultant death. The four other patients had an interval of several years between the attack of glomerulonephritis and the onset of uremia. All four were adults above the age of 35 years. All four had normal complement values and a slow progression of the uremia. These patients at present are still alive.

Healed Nephritis.—Ten patients with healed glomerulonephritis, who had been previously under our observation during their acute symptoms, were subsequently followed at regular intervals for evidence of recurrences and signs of activity. All

these patients showed normal complement levels, and laboratory examination confirmed the absence of activity.

Complement in Relation to Therapeutic Measures.—Chasis and co-workers [20] suggested the use of nitrogen mustards (methyl-*bis* or *tris* [2-chloroethyl] aminohydrochloride) in the treatment of glomerulonephritis, particularly for the nephrotic stage. We examined the serums of three patients receiving nitrogen mustard therapy for diseases other than nephritis. They showed a normal complement content in the serum. One patient in the nephrotic stage was examined before nitrogen mustard therapy and was found to have a low complement content (Table 2). He received nitrogen mustard (0.4 mg. per kilogram of body weight intravenously), his edema cleared, and he lost 22 lb. (10 kg.). Coincidental with the subsidence of the edema the complement titer returned to normal levels. There was neither a decrease in the degree of proteinuria or the blood cholesterol level nor an elevation of serum proteins to correspond with his loss of edema, but the change in complement content was striking.

Corticotrophin (ACTH) and cortisone have been used to induce remissions in the nephrotic stage. We examined two patients receiving corticotrophin therapy

TABLE 2.—*Comparison of Serum Proteins, Complement, Proteinuria, and Edema in Nephrotic Patients With and Without Nitrogen Mustard Therapy (See Text)*

	P. B., M, 32 Yr. Old		N. C., M, 19 Yr. Old	
	Immediately Before Therapy	4 Wk. After 0.4 Mg./Kg. HN_2 I. V.	Nov. 8, 1950	Feb. 27, 1951
Body weight, lb.	192	170	130	141
Albumin, gm./100 cc.	1.56	1.56	1.7	3.0
Globulin, gm./100 cc.	1.80	1.56	1.7	1.0
Daily albumin in urine, gm.	15-22	15-23	22.6	22.4
Complement, units	0.84	1.4	0.24	0.24

and two being given cortisone for diseases other than nephritis. All four showed normal complement levels.

Two patients in the nephrotic stage showed low complement levels before therapy (0.64 and 0.4 unit respectively). After seven days of corticotrophin therapy, diuresis and complete loss of edema occurred. Concurrently the complement levels rose to normal in both patients.

Possible Causes for Low Serum Complement.—A. Anticomplementary Factors: The objection may be made that the low complement content is not due to the binding of complement by the antigen-antibody reaction but to the presence of an anticomplementary substance in the blood stream specific to nephritis. All serums were therefore inactivated and tested for anticomplementary properties in the usual way. No serum was found to be anticomplementary. It would seem to be possible, however, that the anticomplementary substance is destroyed together with the complement during inactivation. The following experiment was, therefore, performed. The serum of a nephritic who showed no complement whatsoever was mixed with an equal amount of serum from a normal person, and the mixture was tested for complement content. At the same time, a part of the serum of the normal control

20. Chasis, H.; Goldring, W., and Baldwin, D. S.: Effect of Febrile Plasma Typhoid Vaccine and Nitrogen Mustard on Renal Manifestations of Human Glomerulonephritis, Proc. Soc. Exper. Biol. & Med. **71**:565, 1949.

was inactivated, mixed with an equal but active part of same serum, and the complement content determined. If both rows showed the same complement content, it could be assumed that no anticomplementary substance was present in the nephritic blood, for it is unlikely that the hypothetical anticomplementary substance is present in the amount to neutralize completely the complement of the nephritic without an excess. It was shown that both rows had identical complement titers, indicating that in all probability no anticomplementary substance was involved.

B. Loss of Complement in the Urine: It could also be considered that complement may be low owing to its loss into the urine with the proteins. That this is not the case is shown by the fact that the lowest complement values are found in the patient with acute nephritis who has relatively little proteinuria, and higher values are usually found in the nephrotic patient with massive proteinuria. In addition, we have seen cases in which the complement content temporarily returned to normal, after nitrogen mustard therapy, as described above, but in which the proteinuria persisted unchanged.

Seifter and Ecker [21] tested the urine of patients with glomerulonephritis in the nephrotic stage for complement. While no complement could be detected in the unconcentrated urine, certain components, especially C'2, were found in small quantities in the urinary protein concentrates. The amounts thus lost, however, are certainly too small to account for the low complement content of the blood, especially if one considers that in cases of a sudden suppression of antibody formation, as in nitrogen mustard medication, the complement content rapidly returns to normal. This is indicative of the great ability of the body to produce large amounts of complement rapidly.

COMMENT

The presented data seem to indicate that during the various phases of active glomerulonephritis the serum complement titer is low. Similar findings were made by Ogawa and Sato [22] in all phases of active nephrotoxic nephritis in rabbits. They reported a precipitous fall of complement within 24 hours or less before signs of acute nephritis appeared. In spite of the immediate selective union [23] of the nephrotoxic serum with the glomerular apparatus, clinical signs and the drop in complement do not occur for approximately seven to 10 days, thus confirming the idea of Kay [4] that the original injection of the nephrotoxic serum serves only to make the kidney antigenic. Homologous antibodies are then formed which react with the kidney in an antigen-antibody reaction after the typical time interval necessary for anaphylactoid reactions. In this experimental nephritis the antigen-antibody reaction is also complement-binding. Similarly, Schwab and colleagues [24] showed that complement falls to low levels in rabbits simultaneously with the appearance of tissue lesions and the disappearance of an injected antigen (bovine gamma globulin

21. Seifter, S., and Ecker, E. E.: Complement and Isohemagglutinins in Urinary Proteins, J. Clin. Invest. **25**:809, 1946.

22. Ogawa, S., and Sato, Y.: Über das Verhalten des Komplementgehaltes im Serum im Laufe der experimentellen Glomerulonephritis, Tr. Soc. path. jap. **28**:212, 1938.

23. Sarre and Wirtz.[5] Pressman.[6]

24. Schwab, L.; Moll, F. C.; Hall, T.; Brean, H.; Kirk, M.; Hawn, C. Z., and Janeway, C. A.: Experimental Hypersensitivity in the Rabbit, J. Exper. Med. **91**:505, 1950.

or albumin) from the circulation. Complement titers increase when free antibodies reappear in the blood stream. We demonstrated previously that antibodies to human kidney are present in the serums of patients with glomerulonephritis. We also showed that the reaction between the antigen and the antibody is complement-binding.[25] It must, therefore, be assumed that the decrease of complement shown in these studies is the consequence of such a reaction, especially since no anticomplementary substances could be found and loss of complement into the urine is insignificant or does not occur. The fall of complement seems to be an indicator of the activity of the disease, i. e., of the binding of antigen and antibody somewhere in the body, most probably in the kidney but possibly throughout the entire capillary network. When other causes for such a fall, e. g., liver diseases, can be excluded, the test seems to be of great diagnostic significance. The return of complement to normal with clinical signs of healing of the nephritis adds further strength to its value. With the technique used, low complement titers may be found for many years in cases of chronic active nephritis and in the nephrotic stage, as well as in some cases of uremia coinciding with clinical signs of activity. Low complement values were found in uremia by several investigators.[26] Others [27] were unable to confirm this work. Kellett and Thomson [15] presented 12 uremic patients with normal complement values. They stated, however, that probably none of these patients had active nephritis.

The slow progression of uremia subsequent to nephritis when complement is normal, in contrast to the rapid progression when complement is low, seems to indicate that immunologic activity is an important factor in the rapid progression of the disease.

The immunologic approach to nephritis as discussed above not only gives a better insight into the mechanism of this disease but also opens new avenues of therapy.

Kay [9] prevented nephrotoxic nephritis by x-ray spray radiation, which suppresses antibody formation. Schwab and co-workers,[24] when injecting bovine albumin and globin in rabbits, were able to suppress antibody formation, the lowering of complement, and the appearance of tissue lesions with the use of nitrogen mustards. Chasis and associates [20] and Taylor and co-authors [28] reported successful treatment of the nephrotic stage with nitrogen mustard, which they used in the assumption that nephritis is an antigen-antibody reaction. We demonstrated (Table 2) that in a patient with the nephrotic syndrome successfully treated with nitrogen mustard the complement content rose to normal at the same time that the patient started to lose his edema fluid. It may, therefore, be assumed that with the suppression of antibody formation by the nitrogen mustard complement was no longer bound by the antigen-antibody reaction, and the level returned to normal. At the same time,

25. Lange, K.; Graig, F.; Oberman, J., and LoCasto, F.: Complement Titres in Different Phases of Glomerulonephritis, Bull. New York Acad. Med. **27**:392, 1951. Lange, K.; Craig, F.; Oberman, J., and LoCasto, F.: Unpublished data.

26. Neisser, E., and Doering, H.: Zur Kenntnis der haemolytischen Eigenschaften des menschlichen Serums, Klin. Wchnschr. **28**:593, 1901. Hedinger, E.: Klinische Beiträge zur Frage der Hämolyse, Deutsche Arch. klin. Med. **74**:24, 1902.

27. Senator, H.: Über die hämolytische Eigenschaft des Blutserums bei Urämie, Klin. Wchnschr. **41**:181, 1904. Rockwood, R., and Beeler, C.: Study of Complement in Serum of Diabetic and Uremic Patients, J. Infect. Dis. **34**:625, 1924.

28. Taylor, R. D.; Corcoran, A. C., and Paige, I. H.: Treatment of the Nephrotic Syndrome with Nitrogen Mustard, J. Lab. & Clin. Med. **36**:996, 1950.

this case demonstrates that the lowered plasma proteins per se cannot be exclusively incriminated as the cause of the nephrotic edema. With normalization of the immunologic mechanism the edema disappeared, while the level of proteins in the blood and in the urine remained unaltered. In contrast, data on another case of the nephrotic stage are shown in Table 2, in which for unknown reasons the plasma proteins rose considerably above the so-called edema level; in spite of this, no weight loss occurred. In this case, however, the immunologic reaction as manifested by the low complement content persisted.

We feel, therefore, from this and a number of similar cases that, as shown previously,[10] an anaphylactoid reaction is at least partly the cause of the edema seen in the various phases of glomerulonephritis.

The influence of corticotrophin and cortisone on antibody formation is still much disputed. Fischel and associates [29] reported that corticotrophin depresses antibody formation. Other authors,[30] however, failed to observe this phenomenon and attributed the therapeutic results of these drugs mainly to changes of tissue reactivity. Since depressed complement values in nephritis rise to normal levels when corticotrophin is given, one may assume that either a suppression of antibody formation similar to that seen with nitrogen mustard therapy or a failure of antibody and antigen union occurs.[31]

The role of complement in the antigen-antibody reaction is still disputed. According to the classical concept of Dale,[32] complement takes no active part in the tissue reaction in anaphylactic states. Recent observations seem to contradict such a purely passive role of complement. Hemolysis of sheep cells will not occur if complement is absent, despite the union of hemolysin with the cells. Witebsky and Neter [33] demonstrated that sheep-cell antiserum with Forssman antibodies is lethal to the chick embryo only if complement is present. Similar findings were reported by Bier and Seiler.[34] Kulka [35] showed that complement is needed for the contraction of the guinea-pig uterus sensitized to Pneumococcus polysaccharide when the antigen is added. If complement not only is an indicator but is also essential for the tissue destruction in a fixed antigen-antibody reaction, certain therapeutic observations may be easily explained. It is well known that measles will induce remissions, at least temporarily, in a certain percentage of cases of the nephrotic syndrome.

29. Fischel, E. E.; LeMay, M., and Kabat, E. A.: The Effect of Adrenocorticotrophic Hormone and X-Ray on the Amount of Circulating Antibody, J. Immunol. **61**:89, 1949.

30. Mirick, G. S.: The Effect of Adrenocorticotrophic Hormone and Cortisone on Antibody Production in Human Beings, J. Clin. Invest. **29**:836, 1950. Hammond, C. W., and Novack, M.: Relation of Adrenal Cortical Steroids to Antibody Release, Proc. Soc. Exper. Biol. & Med. **74**:155, 1950.

31. Since the completion of this report, Burnett and co-authors have reported such a rise in complement in a patient with acute glomerulonephritis receiving cortisone therapy (New England J. Med. **243**:1028, 1950).

32. Dale, H. H.: The Anaphylactic Reaction of Plain Muscle in the Guinea-Pig, J. Pharmacol. & Exper. Therap. **4**:167, 1913.

33. Witebsky, E., and Neter, E.: Primary Serum Toxicity as Demonstrated by Chicken Embryo, J. Exper. Med. **61**:489, 1935.

34. Bier, O. G., and Seiler, E.: Untersuchungen über die Rolle des Komplements bei toxischen Wirkung von Forssman-Antiseren, Ztschr. Immunitätsforsch. u. exper. Therap. **89**:211, 1936.

35. Kulka, A. M.: Studies on Antibody-Antigen Mixtures, J. Immunol. **43**:273, 1942.

Blumberg and Cassady [36] reviewed this subject and added a number of cases of their own. Janeway and colleagues [37] even suggested the induction of measles therapeutically for nephrosis. Erusalimskiy [38] showed that in all cases of measles complement content is low. Ecker and co-workers [39] also demonstrated that a high percentage of patients with measles had a depression of complement, although their results are not as consistent as those of Erusalimskiy. If measles binds complement preferentially during its immunologic reaction, no complement may be available for the nephritic antigen-antibody reaction and the nephrotic edema may thus clear temporarily. Similarly, it was reported [40] that infectious hepatitis may induce remissions in the nephrotic stage. Goldner [19] showed that complement is markedly

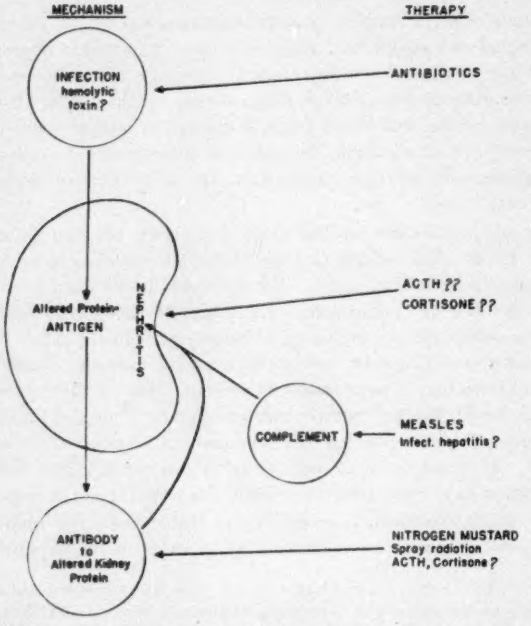

Fig. 3.—A concept of the pathogenesis of glomerulonephritis and the mode of action of therapeutic agents.

depressed in infectious hepatitis (icterus simplex), and a mechanism of preferential use of complement may again be postulated in this coincidence.

36. Blumberg, R. W., and Cassady, H. A.: Effect of Measles on the Nephrotic Syndrome. Am. J. Dis. Child. **73**:151 (Feb.) 1947.

37. Janeway, C. A.; Moll, G. H.; Armstrong, S. H., Jr.; Wallace, W. M.; Hallman, N., and Barness, L. A.: Diuresis in Children with Nephrosis, Tr. A. Am. Physicians **61**:108, 1948.

38. Erusalimskiy, B. M.: Complement Titer in the Blood of Patients with Scarlet Fever and Measles, Med. zuhr. **9**:109, 1939.

39. Ecker, E. E.; Seifter, S.; Dozois, T. F., and Barr, L.; Complement in Infectious Diseases in Man, J. Clin. Invest. **25**:800, 1946.

40. Bergen, H., and Zoole, J.: The Relief of Nephrosis After Hepatitis, J. A. M. A. **145**:228 (Jan. 27) 1951.

These somewhat speculative possibilities are presented in a system, as shown in Figure 3. It represents our present concept of the immunologic features of human glomerulonephritis and the mechanism of action of certain therapeutic measures, a scheme similar to the one suggested by Kay.[4]

The regularity with which depression of complement is found in active glomerulonephritis seems to make complement determinations a practical tool for the diagnosis of such cases, especially when there is clinical doubt of the presence of the disease or the activity.

SUMMARY

The literature on the immunologic features in experimental and human glomerulonephritis is summarized. The presence of serum antibodies to kidney is emphasized in this review. This antigen-antibody reaction is complement-binding in vitro. A technique to determine serum complement (C′) levels is described. Complement levels are shown to be low in all cases of acute and chronic progressive glomerulonephritis. Complement levels return to normal shortly after the clinical signs of activity subside.

In most cases of the nephrotic stage with or without signs of underlying glomerulonephritis complement levels are low. Low complement levels were found in those cases of uremia in which the basic disease was still active.

Successful treatment of the nephrotic stage with nitrogen mustard and corticotrophin (ACTH) is accompanied with a rise in complement to normal levels. The low complement levels are not caused by anticomplementary factors or to loss of complement into the urine.

A theory on the immunologic mechanism of glomerulonephritis is presented and the mode of action of certain intercurrent diseases (measles and infectious hepatitis) is discussed.

Dr. Martin Goldner and Dr. Hyman Katz, of the Veterans Administration Hospital, Brooklyn, cooperated in furnishing us with clinical material.

COMPARISON OF METHODS FOR DETERMINING SENSITIVITY OF BACTERIA TO ANTIBIOTICS IN VITRO

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THE DETERMINATION of the sensitivity of bacteria to antibiotics in vitro has become a common laboratory procedure, the results of which are widely used in defining the range and relative efficacy of antibiotics against different microorganisms. The results of tests for sensitivity are also generally used in the management of the specific therapy of patients with bacterial infections. The sensitivity of any given strain of organism to an antimicrobial agent is generally defined in terms of the concentration of the agent which inhibits the growth of the organism either partially or completely, or which kills the organisms within the time limit of the test.[1] Several methods of determining this value are in common use, and the results obtained with any given strain by the different methods may show wide discrepancies, depending on the details of the method and the choice of the end point.

This difficulty is well known to those familiar with such tests; therefore, in order to achieve some degree of uniformity in the results and interpretations, each laboratory usually selects one method which is best adapted to its needs. Comparisons of values obtained by different laboratories under such conditions are not always justifiable, and the significance of the difference in the results obtained for the same or related strains may be uninterpretable.

It would obviously be of considerable help to the laboratory worker if there were some practical basis for comparing the results of sensitivity tests (and also of antibiotic assays, since they are based on such tests) that are performed by different methods or in different laboratories. Such comparative data would also aid in orienting the physician who is called upon to interpret the results of such tests in the course of selecting and quantitating therapy for the patient. To the clinician the wide discrepancies in the reported results of sensitivity tests are confusing indeed. If such information were available, it might considerably reduce or almost eliminate

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1. Heatley, N. G.: Methods for Measuring the Sensitivity of Microorganisms to Antibiotics, in Florey, H. W., and others: Antibiotics, London, Oxford University Press, 1949, Vol. 1, Chap. 4, pp. 200-214.

the need for routinely carrying out sensitivity tests and would then place the emphasis where it properly belongs, namely, on the importance of establishing an accurate etiologic diagnosis before instituting antibiotic therapy for infections.

In the present study a group of selected strains of bacteria was tested for sensitivity to several antibiotics, each organism being tested with the same antibiotic by a number of different methods simultaneously. In this manner it was possible to reveal the frequency, extent, and direction of the variations in the results obtained with the different methods as applied to the antibiotics that are now in general use. It was hoped, in this manner, also to elucidate some of the factors responsible for the discrepancies.

MATERIALS

Seven bacterial strains and six antibiotics were studied. Details concerning the strains, the methods of maintaining them, and the antibiotics[2] used in this study are given elsewhere.[3] Brain-heart infusion broth, difco,® pH ± 7.4, was used throughout as the liquid medium both for growth of the cultures and for making dilutions of the cultures and antibiotics. The solid medium consisted of heart infusion agar, difco,® pH ± 7.4, with 10% defibrinated equine blood added for the cultures of Streptococcus and Staphylococcus, both of which were hemolytic strains.

METHODS

Four different types of tests for sensitivity were employed: The first involved the electrophotometric measurement of the density of the test organism during growth in serial dilutions of antibiotic; the second employed visual observations of growth in broth containing serial dilutions of antibiotic and also determination of the killing effects by subculture in antibiotic-free medium; the third method involved the incorporation of the antibiotic in solid mediums and observation of growth of the test organisms on the surface, and the fourth involved the diffusion of antibiotic into solid mediums seeded with the test organism. More than one inoculum size was used in some instances; also, readings were made after more than one period of incubation, and end points for partial and complete inhibition, arbitrarily defined, were recorded at these times. Since the details of the tests and the choice of the end points were often crucial, they will be given for each of the methods used.

Turbidimetric Method.—Details of this method and an analysis of the results obtained with the same organisms and antibiotics that are included in the present study have been presented elsewhere.[3] The "complete" and "partial" inhibiting concentrations were arbitrarily defined as the minimum concentrations of antibiotic that produced, respectively, less than one-tenth and less than one-half of the change observed in the percentage of light transmitted through the corresponding culture which contained no antibiotic.

Tube-Dilution Method.—This was adapted from Rammelkamp's method.[4] A 10^{-4} dilution of an overnight culture of the test organism was prepared in broth, and 2% of whole, defibrinated human Group O blood was added. A series of twofold dilutions of the antibiotic through an appropriate range was then made in 0.5-ml. amounts of broth in 100 by 13-mm. culture tubes. An equal volume of the diluted culture suspension was added to each of these tubes; the mixtures

2. The aureomycin was generously supplied by Dr. Stanton M. Hardy, of Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.; the chloramphenicol was furnished by Dr. E. A. Sharp, of Parke, Davis & Company, Detroit 32; the terramycin was provided by Dr. Gladys L. Hobby, of Chas. Pfizer & Company, Inc., Brooklyn 6, and the neomycin was obtained from Dr. E. L. Burbidge, of The Upjohn Company, Kalamazoo 99, Mich.

3. Jackson, G. G., and Finland, M.: Turbidimetric Method for Determining Sensitivity of Bacteria to Antibiotics, J. Lab. & Clin. Med. **38**:289, 1951.

4. Rammelkamp, C. H., and Maxon, T.: Resistance of Staphylococcus Aureus to the Action of Penicillin, Proc. Soc. Exper. Biol. & Med. **51**:386, 1942.

were shaken and then incubated at 37 C. Grossly visible turbidity, hemolysis, or changes in color of the sedimented blood were observed and recorded after 24 and again after 48 hr. of incubation. Complete inhibition was defined as absence of grossly visible turbidity of the broth and absence of alteration in the sedimented erythrocytes. The end point of partial inhibition was chosen as the minimum concentration in which there was faint turbidity, hemolysis, or a suggestion of sedimented organisms, these being markedly less than in the control culture.

Tube-to-Plate Method.—Material from selected tubes at and beyond the end point of partial inhibition in the tube-dilution tests was subcultured at the time of the 24- and 48-hr. readings. A 1-mm. loopful from each of these tubes was streaked on to a sector of an antibiotic-free agar plate that was then incubated for 24 hr., after which it was examined for growth with the aid of an engraver's lens (× 3). The end point for complete inhibition was then defined as the minimum concentration of antibiotic in the original tube which failed to yield discernible growth when subcultured on the antibiotic-free plate. The end point of partial inhibition was similarly defined as the minimum concentration from which the transplanted culture yielded only a small number of isolated colonies.

Plate-Dilution Method.—This method has been used extensively in this laboratory for surveys of the comparative sensitivity of strains of pathogenic bacteria to various antibiotics.[5] A series of culture plates was prepared, each with 10 ml. of agar and twofold dilutions of antibiotic in the appropriate range. Defibrinated equine blood, 10%, was incorporated in the agar for tests with the Streptococcus and Staphylococcus. Each plate was divided into sectors, and a 1-mm. loopful of the test culture was streaked on one of the sectors of each of the series of plates. In comparative tests the inoculum was made directly from the fully grown broth culture and also from suspensions containing 10^{-2} and 10^{-4} dilutions of the seed culture in broth. The plates were examined with the aid of an engraver's lens after 24 and again after 48 hr. of incubation at 37 C., and the growth was recorded at these times. The end point of complete inhibition was defined as the minimum concentration of antibiotic on which there was no detectable growth; the end point for partial inhibition was chosen as the minimum concentration of antibiotic on which there developed either many fine, pinpoint colonies or only a few isolated colonies. Control cultures on antibiotic-free mediums all showed heavy confluent growth.

Medicated-Disk Method.—A number of culture plates, each containing 8 ml. of nutrient agar, were prepared and allowed to harden. A 10^{-2} suspension of the fully grown test organism was then made in melted agar which had been allowed to cool to about 40 C. (10% equine blood was incorporated in this agar for tests with the Streptococcus and Staphylococcus), and 4 ml. of this well-mixed suspension was poured on to the surface of each of the previously prepared agar plates. A series of twofold dilutions of antibiotic in an appropriate range was then made in broth; sterile dry filter-paper disks of 6.35-mm. diameter[6] were each allowed to absorb to capacity one of these dilutions of antibiotic and were placed on the surface of the seeded agar as soon as it had solidified. The disks were spaced far enough apart to prevent both reflection waves from the edge of the dish and overlapping rings of inhibition. The diameter of the clear zone, or of the zone of absent hemolysis in the case of the Streptococcus and Staphylococcus, was measured with the aid of strong transillumination after 24 hr. of incubation. The complete inhibiting concentration was arbitrarily defined as the minimum amount which produced a clear zone, or a zone of absent hemolysis, 10 mm. in diameter, including the diameter of the paper disk. The partial inhibiting concentration was defined as that which produced an 8-mm. zone of complete or partial clearing.

Procedure.—In order to obtain the best comparison and evaluation of the different methods, all the tests done with any given combination of organism and antibiotic, except the medicated-disk method, were set up and carried out simultaneously, using the same seed culture and the same freshly prepared dilutions of antibiotic. From three to seven organisms were tested with a single antibiotic at one time. The tests employing the medicated disks were carried out

5. Frank, P. F.; Wilcox, C., and Finland, M.: In Vitro Sensitivity of Coliform Bacilli to 7 Antibiotics (Penicillin, Streptomycin, Bacitracin, Polymyxin, Aerosporin, Aureomycin, and Chloromycetin), J. Lab. & Clin. Med. **35**:188, 1950.

6. Filter-paper disks were No. 740E, Schleicher and Schull Co.

TABLE 1.—*In Vitro Sensitivity of Seven Organisms to Aureomycin as Determined by Various Methods*

Method	Inoculum*	Hr. of Incubation	Minimum Inhibiting Concentration of Aureomycin, Micrograms per Milliliter: S. typhosa P†	S. typhosa C	S. paratyphi B P	S. paratyphi B C	Sh. dysenteriae P	Sh. dysenteriae C	K. pneumoniae T P	K. pneumoniae T C	B. cereus 5 P	B. cereus 5 C	Streptococcus 98 P	Streptococcus 98 C	Staphylococcus 3 P	Staphylococcus 3 C
A 1 Turbidimetric	10^{-2}	4	0.05	0.4	0.1	0.2	...	...	0.05	0.2	0.001	0.003	<0.05	<0.05	<0.1	<0.1
A 2 Turbidimetric	10^{-2}	6	0.1	0.4	0.2	0.4	0.05	0.4	0.2	0.2	0.003	0.01	<0.05	0.05	<0.1	<0.1
A 3 Turbidimetric	10^{-2}	24	3.1	3.1	3.1	3.1	1.6	3.1	3.1	12.5	0.2	0.2	0.8	0.8	0.2	0.2
A 4 Turbidimetric	10^{-2}	48	25	25	50	50	...	...	50	50	0.8	0.8	12.5	12.5	0.4	0.8
A 5 Turbidimetric	10^{-4}	24	1.6	3.1	3.1	3.1	1.6	3.1	3.1	3.1	0.05	0.05	0.2	0.2	...	...
A 6 Turbidimetric	10^{-4}	48	25	25	50	50	25	100	50	50	0.8	0.8	3.1	3.1	...	...
B 1 Tube dilution	10^{-4}	24	3.1	6.3	6.3	6.3	3.1	6.3	3.1	6.3	<0.1	0.1	0.4	0.8	0.4	0.8
B 2 Tube dilution	10^{-4}	48	12.5	50	25	25	25	25	25	25	0.4	0.8	3.1	6.3	3.1	3.1
B 3 Tube B1 to plate	Undiluted	24	6.3	12.5	12.5	25	6.3	12.5	6.3	12.5	0.1	0.2	1.6	3.1	0.4	1.6
B 4 Tube B2 to plate	Undiluted	24	50	50	100	100	25	50	50	50	0.4	0.8	6.3	12.5	3.1	3.1
C 1 Plate dilution	Undiluted	24	3.1	6.3	6.3	12.5	1.6	6.3	3.1	3.1	0.2	0.4	1.6	1.6	0.8	1.6
C 2 Plate dilution	Undiluted	48	25	25	50	50	25	25	25	25	0.4	1.6	6.3	6.3	3.1	12.5
C 3 Plate dilution	10^{-2}	24	3.1	6.3	6.3	12.5	3.1	6.3	3.1	3.1	0.2	0.2	1.6	1.6	0.4	0.8
C 4 Plate dilution	10^{-2}	48	12.5	25	25	50	12.5	25	3.1	25	0.2	0.2	3.1	6.3	1.6	3.1
C 5 Plate dilution	10^{-4}	24	1.6	3.1	1.6	6.3	1.6	3.1	1.6	3.1	0.1	0.2	0.4	0.8	<0.4	0.4
C 6 Plate dilution	10^{-4}	48	6.3	25	12.5	50	6.3	12.5	1.6	6.3	0.2	0.2	0.4	3.1	0.8	1.6
D Medicated disk	10^{-2}	24	0.8	6.3	0.8	25	<0.8	<0.8	<0.8	50	<0.8	<0.8	<0.8	25	<0.8	6.3
Maximum/Minimum			1,024	128	1,024	512	512	256	1,024	256	512	512	>256	>512	>32	>128
Maximum C/Minimum P			1,024		1,024		2,048		1,024		1,024		512		>128	
Same, excluding A1, A2, and D			32		64		64		32		32		64		64	
Maximum C/P‡			8		32		8		64		4		32		>8	
Same, excluding A1, A2, and D			4		4		4		8		4		8		4	
Maximum 48 Hr./24 Hr.‡			16	8	16	16	16	32	16	32	16	16	16	16	8	8

* Final dilution of seed culture at beginning of incubation except in the plate-dilution and tube-to-plate methods, in which it represents the dilution of seed culture that was taken up on a 1-mm. loop.

† *P* indicates partial, and *C*, complete inhibition, as defined for each method. Leaders indicate test not done.

‡ In the same test.

separately, and all the organisms were tested with all the antibiotics at one time. All concentrations of antibiotics are expressed in micrograms of the active substance per milliliter of medium.

GENERAL RESULTS

It is not feasible to record the results of all the tests with every combination of organisms and antibiotics included in this study. However, sufficient data will be shown in tabular form to illustrate the various features that were brought out by these data. In Table 1, therefore, are shown all the results obtained with one of the antibiotics, namely, aureomycin, and all the seven organisms tested by the various methods. This will serve to illustrate the widest divergences encountered. The results obtained with a single organism, Salmonella typhosa, and all the antibiotics studied are shown in Table 2. The data for a few additional combinations of organisms and antibiotics are given in Table 3.

TABLE 2.—*Sensitivity of Salmonella Typhosa to Various Antibiotics as Determined by Different Methods* *

	Minimum Inhibiting Concentration, Micrograms/Ml.									
	Penicillin		Streptomycin		Chloramphenicol		Terramycin		Neomycin	
Method†	P‡	C	P	C	P	C	P	C	P	C
A 1	6.3	12.5	25	25	0.4	3.1	0.1	0.8	3.1	3.1
A 2	1.6	3.1	25	25	0.8	3.1	0.2	0.8	3.1	3.1
A 3	1.6	12.5	>100	>100	1.6	6.3	0.4	3.1	6.3	12.5
A 4	1.6	12.5	>100	>100	1.6	6.3	0.4	3.1	50.0	50.0
B 1	3.1	6.3	50	50	3.1	3.1	1.6	3.1	6.3	6.3
B 2	6.3	12.5	50	50	3.1	3.1	3.1	3.1	6.3	6.3
B 3	6.3	12.5	50	50	6.3	12.5	1.6	6.3	12.5	12.5
B 4	6.3	12.5	50	100	6.3	50.0	6.3	100.0	12.5	12.5
C 1	6.3	6.3	12.5	25	3.1	3.1	3.1	50.0	6.3	25.0
C 2	6.3	6.3	25	100	3.1	3.1	12.5	50.0	6.3	25.0
C 3	....	....	12.5	25	3.1	3.1	....	....	3.1	6.3
C 4	....	....	25.0	25	3.1	3.1	....	....	3.1	6.3
C 5	3.1	3.1	6.3	12.5	1.6	3.1	....	....	3.1	3.1
C 6	3.1	3.1	6.3	12.5	1.6	3.1	....	....	3.1	3.1
D	25	25	100	100	12.5	25.0	25	100	100	100
Maximum/Minimum	16	8	>16	>8	32	16	256	128	32	32
Maximum C/ Minimum P‖	16 (8)§		>16		128 (16)		1,024 (256)		32	
Maximum C/P‖	8		4		8		16		4	
Maximum 48 hr./ 24 hr.‖	2	2	2	4	1	4	4	16	8	4

* The data for aureomycin are shown in Table 1.
† Designations refer to methods, inocula, and hours of incubation as shown in Table 1.
‡ *P* indicates partial, and *C*, complete inhibition as defined for each method. Leaders indicate test was not done.
‖ For the individual tests.
§ The number in parentheses excludes Methods A1, A2, and D.

In each of these tables the results of the various tests, including the values for complete and partial inhibition, as defined above, are recorded for the different periods of observation and for the different inocula that were used. The variations between the maximum and minimum values for each organism and antibiotic are noted in each instance. The sharpness of the end points is indicated by the ratio of complete to partial inhibiting concentration, as well as by the comparison of the values obtained for any given test at 24 and 48 hr. All the results as illustrated in these tables will, for convenience of presentation, be analyzed first from the point of view of the individual methods and then with respect to the different antibiotics.

TABLE 3.—*Sensitivity of Selected Organisms to Various Antibiotics as Determined in Vitro by Different Methods**

	Minimum Inhibiting Concentration of Antibiotic, Micrograms per Milliliter																			
	Streptococcus 98						Staphylococcus 3				K. pneumoniae T						B. cereus 5			
	Penicillin		Streptomycin		Chlor-amphenicol		Chlor-amphenicol		Terramycin		Penicillin		Streptomycin		Chlor-amphenicol		Penicillin		Chlor-amphenicol	
Method†	P‡	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C
A 1	0.01	0.01	50	100	0.4	0.8	1.6	25	0.05	0.05	50	50	0.2	3.1	0.2	0.8	0.2	3.1	0.8	1.6
A 2	0.01	0.01	50	100	0.8	1.6	3.1	25	0.05	0.2	50	200	3.1	12.5	0.2	0.8	3.1	12.5	0.8	1.6
A 3	0.01	0.01	100	400	1.6	3.1	12.5	25	0.2	0.2	>800	>800	12.5	12.5	0.8	3.1	200	200	3.1	3.1
A 4	0.01	0.01	400	400	1.6	3.1	12.5	50	0.2	0.8	>800	>800	12.5	50	0.8	3.1	>200	>200	3.1	3.1
A 5	0.01	0.01	...	...	...	...	...	...	0.2	0.2	50	50	3.1	3.1	0.8	3.1	3.1	3.1	...	...
A 6	0.01	0.01	...	...	...	...	...	...	0.8	0.8	200	200	3.1	3.1	0.8	3.1	3.1	3.1	...	...
B 1	0.01	0.01	50	50	1.6	1.6	6.3	6.3	0.2	0.2	50	50	3.1	3.1	1.6	1.6	3.1	3.1	1.6	3.1
B 2	0.01	0.01	50	50	1.6	1.6	6.3	6.3	0.2	0.2	50	50	3.1	6.3	1.6	1.6	3.1	3.1	3.1	3.1
B 3	0.01	0.01	100	100	1.6	6.3	12.5	50	0.2	1.6	50	50	3.1	6.3	3.1	3.1	3.1	3.1	3.1	3.1
B 4	0.01	0.01	100	100	1.6	12.5	12.5	25	0.2	1.6	50	50	6.3	6.3	3.1	3.1	3.1	3.1	3.1	3.1
C 1	0.02	0.02	25	25	1.6	3.1	25	200	1.6	6.3	100	100	1.6	3.1	3.1	6.3	3.1	3.1	3.1	6.3
C 2	0.02	0.02	25	25	1.6	3.1	25	200	3.1	6.3	100	100	1.6	3.1	3.1	6.3	3.1	3.1	6.3	6.3
C 3	....	....	25	25	1.6	3.1	...	...	0.8	1.6	..	..	1.6	3.1	3.1	3.1	...	...	3.1	3.1
C 4	....	....	25	25	1.6	3.1	...	...	1.6	3.1	..	..	1.6	6.3	3.1	3.1	...	...	3.1	6.3
C 5	0.006	0.01	25	25	1.6	1.6	3.1	12.5	0.4	0.8	25	25	0.8	0.8	3.1	3.1	0.8	1.6	3.1	3.1
C 6	0.006	0.01	25	25	1.6	1.6	6.3	12.5	0.8	1.6	25	25	0.8	0.8	3.1	3.1	0.8	1.6	3.1	3.1
D	0.1	0.4	>400	>400	100	400	100	100	<0.8	12.5	400	>400	3.1	12.5	6.3	12.5	6.3	25	25	25
Maximum/ Minimum	16	32	>16	>16	256	512	64	32	64	256	>32	>32	64	64	32	16	>1,024	>128	32	16
Maximum C/Minimum P	64 (4)§		>16 (16)		1,024 (8)		128 (64)		256 (32)		>32		256 (64)		64 (8)		>1,024 (256)		32 (4)	
Maximum C/P	4 (2)		4		8		16 (8)		>16 (8)		4 (1)		16 (4)		4		16 (2)		2	
Maximum 48 hr./ 24 hr.‖	1	1	4	1	1	4	2	2	4	4	4	4	2	4	1	1	2	2	2	2

* Data for the sensitivity of each of the organisms to aureomycin are shown in Table 1.
† Designations refer to methods, inocula, and hours of incubation as shown in Table 1.
‡ *P* indicates partial, and *C*, complete inhibition, as defined for each of the methods. Leaders indicate test was not done.
‖ In the same test.
§ The number in parentheses excludes Methods A1, A2, and D.

RESULTS OBTAINED BY THE INDIVIDUAL METHODS

Turbidimetric Method.—Details of the results obtained with this method have been presented elsewhere,[3] so that only the main features need be summarized here. Since this method depends on the measurement of light transmission at a constant wave length, the low solubility of some of the antibiotics and the changes in color of aureomycin and terramycin solutions during incubation may introduce some errors when large concentrations are required. Practically, this was important in the present study only for terramycin, when concentrations greater than 3 micrograms per milliliter were required.

The values for sensitivity obtained with this method were derived from turbidimetric growth curves of the organisms made in the presence of serial concentrations of antibiotic. These curves fell into two general patterns. In the case of penicillin, streptomycin, aureomycin, and neomycin, increases in concentration resulted in progressive delays in initiating growth, but once started the rate of growth and the maximum density attained were approximately the same. With chloramphenicol (chloromycetin®) and terramycin, on the other hand, increases in concentration of the antibiotic produced progressive decreases in the rate of growth and in the final density attained after any given period of incubation.

The size of the inoculum used in these tests influenced the results in various ways. A starting inoculum which gave a final dilution of 10^{-2} of the seed culture permitted early readings which could, in many instances, not be made when the inoculum was 10^{-4} because of the delay in initiating growth from the smaller inoculum. Furthermore, the values for sensitivity to penicillin and streptomycin were much greater when an inoculum of 10^{-2} was used than they were when the inoculum was 10^{-4}. (This, however, was not true in the case of Streptococcus 98 and penicillin.) The results with the other antibiotics were not affected by the size of the inoculum within this range. Wide variations in the values for sensitivity, particularly those for the partial inhibiting concentrations, were also obtained, depending on the period of incubation after which the determinations were made. Sharper end points were usually obtained after 18 hr. or longer.

These features limited the applicability of this method with respect to rapid assay. They account for some of the unusually high values recorded by this method for the sensitivity of some of the organisms to penicillin and streptomycin and also for some of the unusually low values obtained with aureomycin after brief periods of incubation.

Tube-Dilution and Tube-to-Plate Methods.—Both these methods were part of the same tests, the results of the tube-to-plate method more nearly approximating the values for the bactericidal concentrations of the antibiotics. The results for both tests, therefore, will be considered together. The same inoculum, 10^{-4}, was used throughout in the tube-dilution tests.

At any one reading, the sharpest end points in these tests, as judged by the ratio of the complete to the partial inhibiting concentrations, were obtained with penicillin. Streptomycin yielded almost similar results in this respect. Furthermore, most of the values obtained with penicillin in the tube-dilution method at 24 hr. remained unchanged or increased by only twofold (one dilution tube) after 24 hr. of additional incubation in the tube, followed by transfer to the antibiotic-free plate. Here again, streptomycin yielded only slightly less clear results; most of the values

increased two- to four-fold from the minimum partial inhibiting concentration in the tube at 24 hr. to the minimum complete inhibiting concentration in the 48-hr. tube-to-plate method. Aureomycin gave the widest, but most consistent, discrepancies, the differences between these extreme values usually being 8- or 16-fold. The results with chloramphenicol and terramycin were more variable, the corresponding differences usually ranging from 2- to 16-fold for the former and between 4- and 32-fold for the latter.

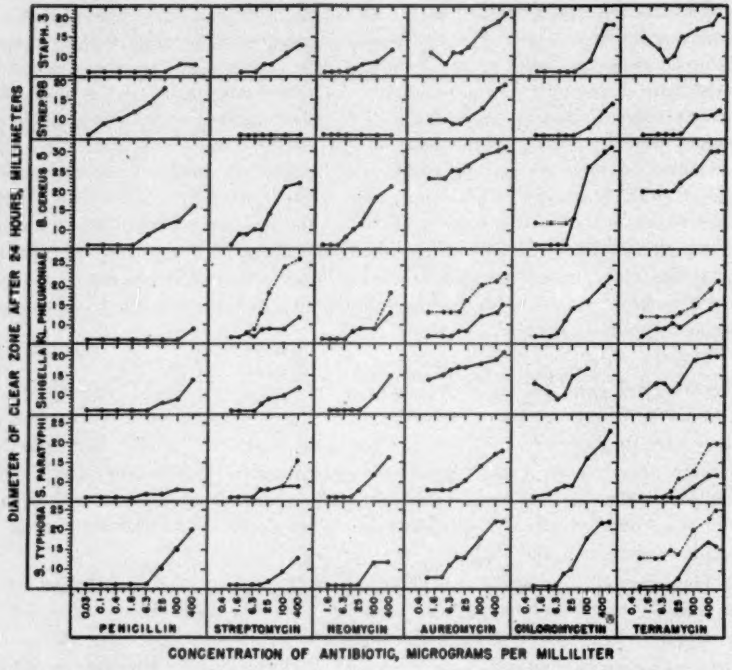
Plate-Dilution Method.—In this test, when undiluted culture was used for the inoculum, penicillin gave the sharpest end points at any one reading, the minimum concentrations for complete and partial inhibition either being the same or varying by only a single dilution. The end points in corresponding tests with chloramphenicol were only slightly less well defined. The results with streptomycin varied markedly in this respect; this variation may have been due, in part, to the emergence of streptomycin-resistant variants in the higher concentrations, since isolated colonies were frequently observed on several plates of the series with no growth discernible on some intervening plates which contained smaller amounts of antibiotic. On the aureomycin-containing plates, growth usually continued after 24 hr. in the region of the limiting concentrations but the end points became more sharply defined after 48 hr. On the plates containing terramycin, it was difficult to read the end points, since marked but incomplete inhibition was observed in most instances over a range of two to more than four dilutions (4- to more than 16-fold), and this was equally true at 24 and 48 hr.

The best measure of the clearness of the end point in this test is obtained by comparing the minimum complete inhibiting concentration at 48 hr. with the minimum partial inhibiting concentration at 24 hr. In this respect most of these values either were the same or varied only twofold in the tests with penicillin and chloramphenicol. The results with streptomycin varied widely in different tests. With aureomycin the ratio of complete inhibiting concentration at 48 hr. to the partial inhibiting concentration at 24 hr. was usually 16 or 32, while with terramycin it usually ranged from four to 32.

The effect of diluting the inoculum down to 10^{-4} also varied considerably with the different antibiotics and with the organism. Most of the organisms appeared to be definitely more sensitive when the smaller inoculum was used. The difference was usually twofold, but occasionally fourfold with penicillin and aureomycin; it was four- to eight-fold with streptomycin and neomycin. In the case of chloramphenicol, however, there was no demonstrable effect from using the dilute inoculum except in the tests with the Staphylococcus, and the latter organism also appeared more sensitive to terramycin when the smaller inoculum was used.

Medicated-Paper-Disk Method.—This part of the study was included because modifications of the medicated-disk method are readily adaptable to use in the clinical laboratory, and some of them have indeed come into wide use. While surface cultures are generally used for this purpose, it seemed more appropriate for the present comparative study to use a measured inoculum well distributed within a thin layer of agar in order to achieve clear definition of the inhibition zones. The readings obtained after 24 hr. of incubation of all the organisms and antibiotics included in this study are shown in the Figure.

It will be seen that, for almost all the combinations of organisms and antibiotics, once inhibiting concentrations of the latter were reached, the diameters of the clear zones of inhibition increased in linear relation to the logarithm of the concentration of antibiotic over a wide range of concentrations. The amount of increase in the diameter of the zone of inhibition that occurred with each twofold increase in concentration of the antibiotic varied considerably, as indicated by the slopes of the lines after they take off from the base line. In general, the most sensitive organisms yielded the steepest slopes.



Results of tests for sensitivity of seven organisms to six antibiotics by a medicated-disk method. Solid lines indicate values for complete clearing, and dotted lines show diameters of zones of partial clearing.

It was also desired, of course, to determine how nearly the results of this method compared quantitatively with those obtained by the other methods with respect to the sensitivity of the organisms. For this purpose it was necessary to define the sensitivity of the organisms in terms of the diameter of the zone of inhibition. The definitions arbitrarily chosen for the minimum complete and partial inhibiting concentrations (namely, those producing clear zones of 10 and 8 mm., respectively) yielded values which corresponded to those obtained by other tests in most instances, but there were some wide discrepancies with several combinations of organisms and antibiotics, as will be seen from some of the values given in Tables 1, 2, and 3. There were also occasional irregular tests, probably due to technical errors, best

illustrated in the test with Staphylococcus and terramycin and with Shigella and chloramphenicol. When such tests were repeated, good linear correlations were readily obtained.

Some of the inhibition zones were fuzzy in outline at 24 hr.; others showed double zones—inner zones with complete clearing and outer ones showing partial growth. The latter are best illustrated in the Figure in the case of terramycin activity on S. typhosa and for the action of aureomycin on the strain of Klebsiella. After 48 hr. of incubation, many of the clear zones observed showed partial growth; this was particularly true of the tests with aureomycin and terramycin.

RESULTS OBTAINED WITH THE INDIVIDUAL ANTIBIOTICS

Penicillin.—Clear end points were obtained with this agent with almost all the methods; the complete and partial inhibiting concentrations were the same in 70% of the tests and varied more than twofold in only 12% of the tests. Furthermore, the results obtained after 24 and after 48 hr. were in agreement in almost every instance. With exclusion of the medicated-disk method and the turbidimetric method employing the 10^{-2} inoculum, the maximum variations in the values for sensitivity of any of the organisms tested with penicillin by all the methods was usually only two- or four-fold.

In the turbidimetric tests with penicillin there were marked discrepancies (16- to 64-fold) between the values obtained with a starting dilution of 10^{-2} and with one of 10^{-4}, except in the case of Streptococcus 98. The results obtained by this method employing the smaller inoculum corresponded more closely with those obtained by all the other methods, except that the paper-disk method in which a large inoculum was used yielded values more nearly approximating those obtained with the larger inoculum in the turbidimetric method. In the plate-dilution method the values obtained with an inoculum of undiluted seed culture were usually twice, and occasionally four times, those obtained when the inoculum was taken from a 10^{-4} dilution of the seed culture.

Streptomycin.—With this agent, too, the end points in the individual tests were usually quite sharp with all the methods. Thus, in 78% of all the individual tests the minimum complete and partial inhibiting concentrations either were the same or varied only by a factor of 2, that is, by a single twofold dilution. There were considerable discrepancies, however, between the values obtained for any given combination of organism and antibiotic when tested by the different methods. In most instances, these differences ranged from 8- to 64-fold and the ratios of the highest values obtained for complete inhibition and the lowest values for partial inhibition usually ranged from 16 to 128.

The size of the inoculum affected the sensitivity of organisms to streptomycin in both the turbidimetric and plate-dilution methods. In the former, however, the differences in values obtained with initial dilutions of 10^{-2} and 10^{-4} of the seed culture were less than those generally noted with penicillin. In the plate-dilution method the values obtained with an inoculum of undiluted culture were usually four or eight times those obtained by employing an inoculum from a 10^{-4} dilution of the same culture. The former, however, corresponded more closely to the results of the tube-dilution tests. The values for sensitivity obtained with the medicated disks were generally greater than those obtained by the other methods except for the turbidimetric assay, which started with a 10^{-2} dilution of culture.

Aureomycin (Table 1).—With this antibiotic the end points for the individual observations in all the tests were quite sharp. The complete and partial inhibiting concentrations, as defined for each test, were the same or varied by only one twofold dilution in 84% of the individual observations, excluding those made with medicated disks. In the latter method the difference between the concentrations of any agent required to produce 8- and 10-mm. zones of inhibition for any given organism varied up to 32-fold. The size of the inoculum, within the range used in these tests, did not have any marked effect on the results, but two- to four-fold differences were noted with the smaller inocula in some instances.

On the other hand, the values for either the partial or the complete inhibiting concentrations increased markedly with increasing periods of observation. Thus, in the turbidimetric method the corresponding values usually increased 8- to 32-fold between six and 24 hr. and another 16-fold between 24 and 48 hr. Likewise, the values obtained at the 24-hr. readings in the tube-dilution method were usually one-eighth or one-sixteenth of the corresponding values obtained by inspection of the plates on which the cultures were inoculated after 48 hr. of growth in the tubes. In the plate-dilution tests the results obtained at 48 hr. were generally four or eight times the corresponding values recorded after 24 hr. of incubation. These discrepancies may be attributable, in part, to the rapid deterioration of the aureomycin on incubation and, in part, to the bacteriostatic or incomplete bactericidal action of the antibiotic in the range of concentrations that were studied.

The maximum differences obtained for the values of the sensitivity of any organism to the aureomycin, as determined by the various tests, were as much as 512- to 1,024-fold. Even excluding the extreme values obtained with the medicated disks or with the short incubation period in the turbidimetric method, these values showed as much as 64- to 128-fold variations.

Chloramphenicol.—The results obtained with this agent by the different methods were highly consistent, resembling, in this respect, the findings with penicillin. Except for the medicated-disk method, sharp end points were generally obtained in the individual tests, the complete and partial inhibiting concentrations being the same or differing by only a single twofold dilution in more than three-fourths of the tests. Furthermore, the end points were usually reached early, there being only a two- or four-fold increase in the values obtained in the turbidimetric tests between six and 48 hr. The values recorded at 24 and 48 hr. were identical in 82% of all tests and differed only by a single dilution in almost all the others. Decreasing the size of the inoculum in the plate-dilution method had no significant effect except with the Staphylococcus, for which the values obtained with an inoculum of undiluted culture were 8 to 16 times higher than those obtained with an inoculum from the 10^{-4} dilution of the same culture. In the turbidimetric tests only the strain of Klebsiella was tested with both 10^{-2} and 10^{-4} dilutions of culture, and the results were essentially the same.

With exclusion of the medicated-disk method, the greatest variations between the maximum value for complete inhibition and the lowest value for the partial inhibiting concentration for any organism was usually only two- or four-fold, although the tests with the Staphylococcus and isolated tests with other organisms showed wider discrepancies up to 32- or 64-fold. The paper-disk method usually yielded high values at 24 hr.; even the concentrations required to produce 8-mm. clear zones

at that time (here defined as partial inhibition) were generally higher than the values obtained for the complete inhibiting concentrations by other methods.

Terramycin.—With this antibiotic, the end points in the individual tests at any given time were somewhat variable. In about half of the observations the complete and partial inhibiting concentrations either were the same or varied only twofold; in an additional 40% of the individual readings the ratio of complete to partial inhibiting concentrations was four or eight, and in the remaining tests, most of them with the Staphylococcus, there were even wider discrepancies. In some of the plate-dilution tests it was possible to demonstrate viable organisms from several dilutions beyond the one which produced marked inhibition even after 48 hr.

In the turbidimetric method the values obtained for both partial and complete inhibition by terramycin usually increased as much as 8- or 16-fold from the 6- to the 24-hr. reading. The changes in light transmission that result from changes in the color of this antibiotic when concentrations of 3 micrograms or more per milliliter are used have already been mentioned. Between 24 and 48 hr. the values obtained by this and all other methods either remained unchanged or increased only twofold in 85% of the tests, greater increases being observed chiefly in the tests with the Staphylococcus.

The effect of reducing the size of the inoculum on the sensitivity to terramycin was studied only with the Staphylococcus (Table 3). There was no important effect in the turbidimetric method. However, in the plate-dilution method the sensitivity of this organism increased fourfold when the inoculum was reduced from the undiluted seed culture to the 10^{-4} dilution.

Paper disks medicated with terramycin often yielded zones of inhibition which were not clearly outlined at 24 hr. and became even less distinct at 48 hr. The readings at 24 hr. by this method correlated irregularly with those obtained by other methods but more nearly approximated those obtained in the plate-dilution tests.

The maximum variations in the values for the sensitivity of individual strains to terramycin obtained by all methods was 4- to 16-fold for the partial inhibiting concentration and 16- to 32-fold for the complete inhibiting values. The ratios of the maximum values for complete inhibition to the minimum values for partial inhibition ranged between 8 and 256 and was 32 in most instances.

Neomycin.—Observations with this agent were made by all methods only with S. typhosa (Table 3), and all seven organisms were tested by the medicated-disk method (Figure). Tests were also made by the tube-dilution, tube-to-plate, and plate-dilution methods with the four strains of Gram-negative bacilli. Except in the turbidimetric method, the results at 24 and 48 hr. were the same in all these tests. The medicated disks yielded much higher values than those obtained by the tube- or plate-dilution methods. In the plate-dilution method the organisms were 4 to 16 times more sensitive when the inoculum was obtained from the 10^{-4} dilution of culture than they were when the undiluted culture was used.

COMMENT

By way of summary and for purposes of comparison there are shown in Table 4 the ranges of values obtained by all the methods employed for the sensitivity of each of the seven organisms to the five antibiotics that are in general use. For simplicity, only the complete inhibiting concentrations, as defined for each of the methods, are

given for a single period of observation, namely, after 24 hr. of incubation. These ranges are compared, in each instance, with the values obtained for complete inhibition by the tube-dilution method, employing visual reading after the same period of incubation. The latter method is the one which is probably most widely used in clinical laboratories.

In extensive studies in this laboratory, however, the plate-dilution test, employing an inoculum of undiluted culture and an incubation period of 48 hr., has been used recently.[7] In the present study the values obtained by the latter method were usually the same or one dilution higher than those obtained by the 24-hr. tube-dilution methods in the tests with penicillin, streptomycin, and chloramphenicol, but they were 4- to 16-fold higher with aureomycin and 16 to 32 times greater in tests with terramycin. The 24- and 48-hr. readings in the plate-dilution tests were essentially the same with all antibiotics except aureomycin. The discrepancy between the results of the tube-dilution and the plate-dilution methods for terramycin was equally present at the 24-hr. readings, whereas with all the other antibiotics, includ-

TABLE 4.—*Sensitivity of Seven Organisms to Five Antibiotics* *

	Minimum Complete Inhibiting Concentration, Micrograms per Milliliter									
	Penicillin		Streptomycin		Aureomycin		Chloramphenicol		Terramycin	
Organism	Range†	Tube	Range	Tube	Range	Tube	Range	Tube	Range	Tube
S. typhosa	3.1-25	6.3	12.5-100	50	3.1-25	6.3	3.1-25	3.1	3.1-100	3.1
S. paratyphi B	50->400	50	50-800	200	3.1-25	6.3	6.3-25	6.3	3.1-50	3.1
Sh. dysenteriae	1.6-100	1.6	3.1-100	6.3	<0.8-6.3	6.3	0.8-6.3	1.6	0.8-50	1.6
K. pneumoniae	25->400	50	0.8-12.5	3.1	3.1-50	6.3	1.6-12.5	1.6	3.1-6.3	6.3
B. cereus 5	1.6->200	3.1	3.1-400	50	0.05-0.4	0.1	3.1-25	3.1	0.2-3.1	0.2
Streptococcus 98	0.01-0.4	0.01	25->400	50	0.2-25	0.8	1.6-400	1.6	0.4-100	0.4
Staphylococcus 3	0.8-800	0.8	50-200	50	0.2-6.3	0.8	6.3-200	6.3	0.2-12.5	0.2

* Range of values for complete inhibiting concentration obtained at 24 hr. by all methods, as compared with values obtained by tube-dilution method.

† The highest of these values for penicillin were obtained in each instance by the medicated-disk method, or by the turbidimetric method employing a 10^{-2} dilution of culture, or by both of these methods.

ing aureomycin, the two tests gave results at 24 hr. which tallied within a single twofold dilution.

The wide range of values for sensitivity obtained for each combination of organism and antibiotic is brought out quite strikingly in Table 4. These values diverge even more widely when partial as well as complete inhibition and various periods of incubation are also included in these ranges. The same mediums were used throughout the present study, but variations in the medium may introduce still further discrepancies.[8] In some instances, to be sure, the extreme values within any given range were due to results obtained with only one of the tests or at the most widely separated periods of incubation, or they may have been due in some instances to technical aberration in the individual tests. Nevertheless, these data emphasize clearly the importance of defining all the conditions under which tests for sensi-

7. Frank and others.[5] Frank, P. F.; Wilcox, C., and Finland, M.: In Vitro Sensitivity of Bacillus Proteus and Pseudomonas Aeruginosa in 7 Antibiotics (Penicillin, Streptomycin, Bacitracin, Polymyxin, Aerosporin, Aureomycin and Chloromycetin), J. Lab. & Clin. Med. **35**:205, 1950.

8. Joslyn, D. A., and Galbraith, M.: A Turbidimetric Method for the Assay of Antibiotics, J. Bact. **59**:711, 1950.

tivity are performed and of appreciating the limitations of all in vitro tests for sensitivity.

At least three factors have been demonstrated in the present study to influence profoundly the values which are assigned to the sensitivity of any organism to a given antibiotic. These are the size of the inoculum, the period of incubation, and the choice of either partial or complete inhibition. In addition, as already noted, the choice of the medium may also have an important effect, but only the difference between a fluid and a solid medium was studied here. It was shown, however, that these factors may have markedly different effects depending on the antibiotic that is being tested, and, with some of these agents, they may also vary in their effects on different organisms.

No attempt has been made in this study to define the reliability of the individual methods that were used. In extensive experiences with the tube-dilution and plate-dilution methods it has been possible to duplicate results in repeated tests with almost all the antibiotics within the limits of error of these methods, that is, within a single twofold dilution. With aureomycin, however, because of its lability under the conditions of these tests, the results of repeated tests vary somewhat more widely. A more limited experience with the turbidimetric method has indicated that results obtained by that method are equally reliable, provided that the initial dilution of culture and the period of incubation are kept constant. None of the methods, however, lend themselves to accurate quantitation, although reliability and accuracy can perhaps be increased to some extent by multiple replications, which are generally impractical.

There has been a tendency among many clinicians, and perhaps some laboratory workers as well, to place undue reliance on the results of tests for sensitivity and of assays of antibiotic concentrations without adequate appreciation of their limitations. This reliance may have stemmed largely from the experiences with penicillin, and perhaps also with streptomycin, in which relatively uniform results can be obtained with most of the methods that are commonly used. Even with these agents, however, one can easily be misled in the case of some strains by employing slight variations of the methods, such as alterations in the size of the inoculum. In the case of aureomycin and terramycin the results of sensitivity tests can be most deceiving, since one may readily obtain either high or low values within wide limits merely by the proper choice of the conditions of the test.

The frequent lack of correlation between the results of the test for sensitivity of an organism to a given antibiotic in vitro and the therapeutic effect of that agent in experimental or clinical infections with the same organism can thus be readily interpreted. The animal or human tissues and body fluids merely present other sets of conditions in which the medium, the number of infecting organisms, and the concentration of antibiotic in contact with the organism may vary widely and may contrast markedly with the conditions selected for the in vitro tests. Indeed, the differences between therapeutic results in human clinical disease and in experimental infections of animals are also interpretable on the same basis. The data presented here, however, offer no clue concerning the mechanisms of action of the various antibiotics which are responsible for the wide differences that have been noted.

It is not meant to imply that tests for sensitivity of bacteria to antibiotics have no value whatever. It is more proper to infer from these results that they can be most properly used as a general qualitative guide to distinguish susceptible from

nonsusceptible organisms. It is also possible, by standardizing the details of the method, to obtain relative values which may be useful in visualizing the relative susceptibility of different organisms, or of different strains of the same species to any given antibiotic. In so doing it must be appreciated that, with the variability in the growth requirements of different bacteria, the choice of the medium and of other conditions may profoundly influence even the relative values as between different bacterial species.

The application of a single method in this manner to large numbers of strains of a variety of different pathogenic bacteria has yielded information that has proved useful for clinical purposes.[7] The data thus obtained have indicated, for example, the fact that strains of some species of organisms, such as pneumococci,[9] gonococci,[10] or Group A streptococci,[11] are more or less uniform in their susceptibility to any given antibiotic, while strains of others, notably staphylococci [12] and enterococci,[11] may vary widely in this respect. It thus becomes most important to place the emphasis on the determination of the true bacterial incitant of any given infection. Antibiotic therapy can then be selected intelligently for most infections without resort to tests for sensitivity except when one is dealing with causative organisms different strains of which have been shown to exhibit wide variations in their susceptibility to the antibiotics which are under consideration.

SUMMARY AND CONCLUSIONS

The sensitivity of seven organisms to six antibiotics was tested by several methods, including various modifications of the methods that are most widely used.

Wide ranges of values were obtained for the minimum inhibiting concentrations of any one antibiotic for any given organism, depending on the method used. In particular, these values were shown to be influenced profoundly by the size of the inoculum, the period of incubation, and the choice of complete or partial inhibition as the end point of the tests. These factors affected the results differently, depending on the combination of organism and antibiotic that was being tested.

The value and limitations of the results of tests for sensitivity of bacteria to antibiotics are discussed, particularly with respect to their clinical application.

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9. Jackson, G. G.; Gocke, T. M.; Wilcox, C., and Finland, M.: In Vitro Susceptibility of Pneumococci to 7 Antibiotics (Penicillin, Streptomycin, Bacitracin, Polymyxin, Aerosporin, Aureomycin and Chloromycetin), Am. J. Clin. Path. **20**:218, 1950.

10. Gocke, T. M.; Wilcox, C., and Finland, M.: Antibiotic Spectrum of the Gonococcus, Am J. Syph. **34**:265, 1950.

11. Finland, M.; Wilcox, C., and Frank, P. F.: In Vitro Sensitivity of Human Pathogenic Strains of Streptococci to 7 Antibiotics (Penicillin, Streptomycin, Bacitracin, Polymyxin, Aerosporin, Aureomycin and Choloromycetin), Am. J. Clin. Path. **20**:208, 1950.

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INFECTION DUE TO CHROMOBACTERIA

Report of Eleven Cases

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INFECTION of human beings by chromobacteria is a relatively rare occurrence. Hence, it has been of considerable interest in the past six months to observe in this hospital 11 cases of infection by these organisms, one of which had a fatal termination with bacterial endocarditis.

The chromobacteria have been described as nonsporulating, aerobic bacilli, usually motile and usually Gram-negative, producing yellow, red, or violet pigment which is generally insoluble in water. The bacteria are ordinarily saprophytic and are commonly found in water, soil, and sewage. They grow abundantly and with ease on ordinary mediums, and their most arresting feature is the pigment production, which occurs only in the presence of oxygen, usually only on solid mediums at suitable temperatures, and is most abundant on the primary culture.[1] The chief member of the group producing a violet pigment is Chromobacterium violaceum, that of the group producing pink or red pigment is Chromobacterium prodigiosum (Serratia marcescens), and that of the group producing yellow or orange pigment is Chromobacterium aquatilis. The organisms have been isolated from many sources since Bizio [2] first recorded his observations on S. marcescens in 1823. This young Italian pharmacist delightfully and accurately explained the outbreak of red-colored polenta or "bleeding mush," as it was called by the fearful peasantry of the period. In this communication he first described the causative organism, which he thought to be a fungus, giving it the name Serratia marcescens. It is of historical interest that this description was written one year after the birth of Louis Pasteur.

The clinical experience to be described was with infection by members of the pink- or red-pigment-producing group, presumably Chr. prodigiosum (or S. marcescens).

Chromobacteria have been considered essentially nonpathogenic in man, but there are scattered reports describing the potential pathogenic properties of the organisms. Bertarelli [3] in 1903 reported that intraperitoneal injection of small numbers of the

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1. Topley, W. W. C., and Wilson, G. S.: Principles of Bacteriology and Immunity, revised by G. Wilson and A. Miles, ed. 3, Baltimore, Williams & Wilkins Company, 1946, pp. 631-641.

2. Bizio, B.: The Phenomenon of the Red Colored Polenta, Biblioteca italiana o sia Giornale de lettera, scienze e arti **30**:275, 1823.

3. Bertarelli, E.: Untersuchungen und Beobachtungen über die Biologie und Pathogenität des Bacillus prodigiosus, Zentralbl. Bakt. **34**:193 and 312, 1903.

organisms in mice and rabbits was lethal. Guinea pigs and rabbits were less susceptible. In 1933 Rosahn and Hu [4] also studied the pathogenicity of several strains in various animals, their results confirming the earlier work. Woolstein and Meltzer [5] found, in 1913, that intrabronchial insufflation of broth cultures of S. marcescens resulted in hemorrhagic and necrotic pulmonary lesions, leading to bacteremia and death in dogs. In 1905 Woolley [6] first described naturally occurring disease caused by a Chromobacterium, when he recovered the organism from dying and dead water buffalos which he studied in the Philippines.

Human infection by chromobacteria was first reported in 1913 by Woodward and Clark,[7] who described a patient with chronic, nonfatal pulmonary disease whose red-tinged sputum was not bloody as expected but was harboring swarms of bacteria which on culture proved to be red-pigmented Gram-negative bacilli. Since then, other isolated reports have entered the literature, including cases of post-lumbar-puncture meningitis,[8] pneumonia,[9] fatal wound infection,[10] chronic, fatal granu-

Clinical and Bacteriological Data in Eleven Cases of Infection Due to Chromobacteria

Case No.	Age, Yr.	Sex	Underlying Disease	Catheterization	Cystoscopy	Date Chromobacterium Isolated	Where Isolated	Sensitivity					
								Penicillin	Streptomycin	Aureomycin	Chloramphenicol	Terramycin	Polymyxin B
1	50	M	Chronic pyelonephritis	+*	+	9-29-50	Urine	0*	0	0	0	0	..
2	67	M	Benign prostatic hypertrophy	+	..	10- 2-50	Urine	..	..	..	..	..	..
						10-16-50	Blood	0	0	0	0	0	0
3	45	M	Nephrolithiasis	+	+	10- 9-50	Urine	0	0	0	0	0	..
4	78	M	Benign prostatic hypertrophy	+	..	10-12-50	Urine	0	0	0	0	0.	..
5	45	F	Pelvic relaxation with plastic repair	+	..	10-27-50	Urine	0	0	0	0	0	..
6	29	F	Cystitis with regional ileitis..	+	..	11- 4-50	Urine	0	0	0	0	0	..
7	44	F	Pelvic relaxation with plastic repair	+	+	12-21-50	Urine	0	0	0	0	0	..
8	59	M	Chronic pyelonephritis	+	+	1- 9-51	Urine	..	..	..	..	..	..
						1-12-51	Blood	0	0	0	0	0	..
9	69	M	Cystitis	+	+	2-16-51	Urine	0	0	0	0	0	..
10	75	M	Carcinoma of rectum.....	+	..	2-20-51	Urine	0	0	0	0	0	..
11	72	M	Urinary-tract infection	+	..	2-21-51	Urine	0	0	0	0	0	..

* Plus signs indicate procedure was performed. Zeros show lack of sensitivity of isolated organisms to antibiotics.

4. Rosahn, P., and Hu, C.: Pathogenicity of Serratia Marcescens (Bacillus Prodigiosus), Proc. Soc. Exper. Biol. & Med. **30**:1326, 1933.

5. Woolstein, M., and Meltzer, S.: Lesions Produced by Intrabronchial Insufflation of Bacillus Prodigiosus, Science **38**:452, 1913.

6. Woolley, P. G.: Bacillus Violaceus Manilae (A Pathogenic Organism), Bull. Johns Hopkins Hosp. **16**:89, 1905.

7. Woodward, H., and Clark, K.: A Case of Infection in Man by the Bacterium Prodigiosum, Lancet **1**:314, 1913.

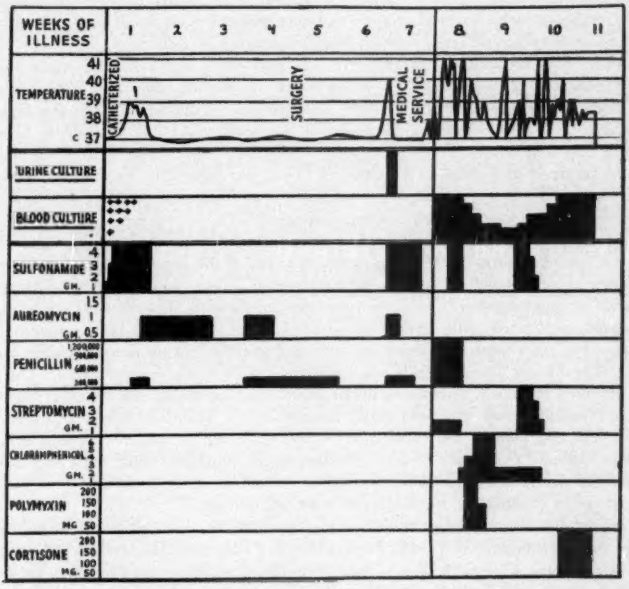
8. Aronson, J., and Alderman, I.: The Occurrence and Bacteriological Characteristics of Serratia Marcescens from a Case of Meningitis, J. Bact. **46**:261, 1943.

9. Aitoff, M.; Dion, M., and Dobkevitch, H.: Bacillus prodigiosus pathogène pour les animaux: Endotoxine et réaction de Shwartzman, Compt. rend. soc. biol. **123**:375, 1936.

10. Schattenberg, H., and Harris, W. H.: A Definite and Unique Occurrence of Rapidly Fatal Infection Caused by Bacillus Violaceus Manilae, J. A. M. A. **117**:2069 (Dec. 13) 1941.

lomatous skin disease,[11] and others.[12] There is also one report by Pangálos [13] in 1929 of possible bacteremia attributed to yellow-pigmented chromogenic bacteria. The disease apparently was transient and nonfatal.

The group of patients reported on here is of interest in that the infection was recognized and the organism first recovered in all the cases after some type of urinary-tract manipulation. This included catheterization, cystoscopy, and genitourinary surgical treatment. These procedures did not take place in the same clinic; different operators were involved, and various methods of sterilization of instruments were employed. An epidemiological study failed to locate the source of this unusual organism.



Clinical course in a fatal case of Chromobacterium sepsis with bacteremia and endocarditis. Polymyxin B was used in these therapeutic trials.

In all cases the organism was present in the urine in large numbers and in two cases was recovered from the blood. Bacteremia was transient in one; fatal endocarditis was present in the other. There was relatively little generalized toxicity,

11. Black, M. E., and Shahan, J.: Bacillus Violaceus Infection in a Human Being, J. A. M. A. **110**:1270 (April 16) 1938. Soule, M. H.: A Study of 2 Strains of B. Violaceus Isolated from Human Beings, Am. J. Path. **15**:592, 1939.

12. Paine, T.: Illness in Man Following Inhalation of Serratia Marcescens, J. Infect. Dis. **79**:226, 1946. Schutz, F., and Laun, H.: Über einen seltenen Bakterienbefund im menschlichen Abszesseiter, Zentralbl. Bakt. **129**:124, 1933.

13. Pangalos, G.: Sur un bacille chromogène isolé par hémoculture, Compt. rend. Soc. biol. **100**:1097, 1929.

even in the patient with endocarditis, and few local symptoms were attributable to the Chromobacterium even when large numbers of organisms were present in the urine.

An opportunity was presented by the patient with endocarditis to study the course of the infection in detail, both clinically and at autopsy. Prior to the emergence of the bacteremia, his illness was quite typical of that of the other patients and is reported at some length.

REPORT OF CASE

CASE 2.—E. N., a 67-yr.-old retired pipe fitter, entered the medical service on Oct. 11, 1950, complaining of chills, fever, and malaise. Arteriosclerotic heart disease, with mild failure, and moderately advanced prostatic hypertrophy had been present for some time. Two months prior to the last admission acute urinary retention developed, and continuous catheter drainage was instituted in preparation for surgical removal of the prostate. A fever developed, and sulfonamides and aureomycin were administered without a preliminary urine culture.

One month later retropubic prostatectomy was performed and was complicated by a brief period of hypotension and oliguria. His postoperative course was otherwise unremarkable. He had been given routine prophylactic treatment with aureomycin and penicillin during this sojourn in the hospital. Ten days before his admission he had fever and chills, thought to be due to a urinary infection, and Gram-negative, chromogenic bacteria were isolated from the urine. The temperature returned to normal in a matter of hours, but penicillin, aureomycin, and sulfonamides were administered for several days. He had just arrived at home after this incident when fever returned, and he was readmitted to the hospital.

Physical examination revealed a febrile, acutely ill, aged man, who was having a chill. The skin was hot and moist, and there was moderate cyanosis of the lips and nail beds. No petechiae or other evidence of embolic phenomena were discovered. There was moderate bilateral cervical and inguinal lymphadenopathy. The neck veins were moderately distended. There was evidence of emphysema, associated with dulness to percussion and diminished breath sounds over the lower lobe of the right lung. The heart was enlarged slightly, but no murmurs were heard, and the sounds were distant. The pulse rate was rapid and the rhythm grossly irregular. The abdomen was flat and firm, there was tenderness in the right upper quadrant, and the liver was palpable 3 to 4 fingerbreadths below the right costal margin. There was no costovertebral-angle tenderness; the spleen and kidneys were not palpable, and there was no evidence of free fluid. Bowel sounds were normal. There was a recent, healing lower abdominal scar, with only slight local tenderness. The genitalia and rectum were normal, except for some tenderness over the prostatic bed. No edema or clubbing of the fingers or toes was present.

The hemoglobin concentration was 12.4 gm. per 100 cc.; the white-blood-cell count was 17,600 per cubic millimeter, with 91% neutrophils, and the corrected erythrocyte-sedimentation rate (Wintrobe) was 47 mm. per hour. Small amounts of protein were present in the urine, and the sediment contained 10 to 12 white blood cells per high-dry field. Sputum culture revealed a heavy growth of Staphylococcus pyogenes var. albus. Large numbers of Escherichia coli were present in the urine. The blood urea was 77 mg. per 100 cc., and an electrocardiogram showed a probable auricular flutter, with 2 to 1 block. A Gram-negative chromogenic organism similar to the organism isolated from the urine 10 days previously was recovered from the blood. A chest roentgenogram showed pulmonary emphysema, with no evidence of parenchymal lesions, and a flat plate of the abdomen was normal. A diagnostic pleural tap on the right showed only a few cubic centimeters of yellow fluid, which was sterile.

Antibiotic therapy was instituted after these examinations, and this and future therapy is noted in detail on the Chart. The fever during the hospital stay was septic, and there were severe daily chills and heavy sweats. Clinical trial bore out laboratory predictions regarding the insensitivity of the organism to antibiotics. The only evidence of improvement came while chloramphenicol (chloromycetin®) was being given in 6-gm. daily doses, but the temperature did not remain normal, and at no time was the daily blood culture free of organisms, although during this period the colony count fell from 200 to 5 to 10 per cubic centimeter. Transfusions were given at intervals. When treatment failed with the usual antibiotics, both separately and in combination, a therapeutic trial with polymxin B was started, even though the organism

was highly resistant to the agent in vitro; this antibiotic also had no effect on the course of the disease. A lumbar puncture was done during this period, and the spinal fluid was noted to be normal chemically and sterile.

The patient remained in remarkably good condition until the last week, when progressive deterioration began. Cortisone was given, but this had little effect, and the patient's condition went downhill rapidly. Throughout this time no change was noted on physical examination, except for the gradual accumulation of a small amount of pleural fluid. Daily examinations for petechiae failed to reveal their presence, and no change in his cardiac status, except for occasional irregularities of rhythm, was noted. No murmurs were ever heard. On the 20th hospital day the patient was found to be jaundiced, and, on the 21st day, some 10 weeks after the onset of his original urological disturbance, he died. The clinical diagnosis was Chromobacterium urinary-tract infection with bacteremia. It was believed that bacterial endocarditis was almost certainly present. Arteriosclerotic heart disease was a complicating factor, but evidence of valvular damage was never obtained.

Autopsy showed two large, friable vegetations on the tricuspid valve, with multiple septic emboli to the lungs, mild pyelonephritis, and severe acute hepatitis. Chromobacterium organisms were isolated from the heart blood.

The vegetation was composed of laminated, amorphous, slightly granular material, surrounded by a zone of inflammatory exudate and a necrotic zone extending onto the base of the tricuspid leaflet. The other valves and the myocardium were not remarkable. Microscopic section of the lungs showed areas of necrosis and hemorrhage and polymorphonuclear infiltrates through the upper, right middle, and lower lobes. An atrophy of liver cells around the central vein was demonstrated. Many of the hepatic cells were bile-stained. The periportal fibrous tissue was heavily infiltrated with lymphocytes and polymorphonuclear cells. The hepatic cords were widely dilated, and the sinusoids were filled with pink granular material and some necrotic cells. Focal areas of lymphocytic infiltration were seen throughout the kidneys, associated with tubular atrophy and an increase in interstitial fibrous tissue. The uninvolved areas, glomeruli, and tubules were not remarkable, except for a loculated cyst near the cortex in one section.

The anatomical diagnoses were acute endocarditis of the tricuspid valve, septic emboli of the lungs with abscess formation, probable septic hepatitis, and chronic cystitis and pyelonephritis.

Bacteriological Study.—The Chromobacterium which was isolated from the blood on many occasions grew abundantly on blood and plain agar and in liver-digest broth. It was similar to the strains isolated from the blood in one other case and from the urine in 10 other cases. The colonies were small, rounded, and convex and had smooth surfaces. The organism was aerobic and grew well at temperatures of 26 to 37 C. Pigment production was maximal at incubation below 30 C. Individual colonies grown on agar varied from colorless to bright red, probably depending upon the colony age. The pigment was insoluble in water and readily soluble in alcohol. The organism fermented glucose, sucrose, mannitol, and inositol without the formation of gas. Two of the strains also fermented lactose.

When injected intraperitoneally in the mouse in doses varying from light to heavy suspension of a 24-hr. broth culture (10^{0} to 10^{-3} dilution), the organism caused death in six to 36 hr. The Chromobacterium was recovered in each case from the peritoneum and the heart blood. For more detailed studies of animal pathogenicity, reference is made to the work of Aronson and Alderman[8] and Rosahn and Hu.[4] The serum of the patient with endocarditis agglutinated the homologus organism at a titer of 1:32 with a negative control. Several workers[14] had previously demonstrated the development of specific agglutinins in animals after the injection of living or dead chromobacteria.

Sensitivity studies, using blood-agar plates containing antibiotics, demonstrated remarkable resistance on the part of the Chromobacterium isolated from these 11 patients. The organism isolated from the patient with endocarditis grew in the presence of 100 micrograms of polymyxin B, 250 micrograms of terramycin, 50 micrograms of chloramphenicol, and 50 micrograms of

14. Hefferan, M.: Agglutination and Biological Relationship in the Prodigiosus Group, Zentralbl. Bakt. **61**:553, 1906. Schad, G.: Die Agglutination als ein Hilfsmittel zur Identifizierung farblos gewordener Prodigiosuskeine, Arch. f. Hyg. **104**:99, 1930. Paraschivesco, Z.: Sur l'antigène somatique "complet" renfermé dans le Micrococcus prodigiosus, Compt. rend. Soc. biol. **121**:175, 1936.

streptomycin, per cubic centimeter. It was totally insensitive to penicillin and only moderately sensitive to sulfonamides. Various combinations of these agents were also investigated, and all proved inefficacious in enhancing the antimicrobial effects. Strains isolated from the other 10 patients were not studied in the same detail, but all were highly resistant to this group of antimicrobial agents.

COMMENT

The isolation of a red-pigment-producing Chromobacterium from the urine of human beings was of interest, at first, as a curious clinical observation. Later, the repeated occurrence of urinary-tract infection by this organism, with bacteremia in two patients and death in one, indicated the potential clinical importance of this group of bacteria.

In the absence of dissemination the disease was not severe. Few local symptoms were present. Instrumentation of the urinary tract had been performed in every case, and the Chromobacterium probably was introduced by these procedures. An epidemiological study failed to reveal the route of infection in detail.

That so many cases of urinary-tract infection by this unusual organism should have been observed was not surprising, since the obstructed and instrumented urinary passages are fertile soil for the multiplication of bacteria that are not commonly the cause of disease elsewhere. A contributing factor was the use of multiple antibiotics, which eliminated all the usual organisms that are responsible for infection of these organs and permitted the ready implantation of the highly antibiotic- and sulfonamide-resistant Chromobacterium.

Similar invasion of various organs by bacteria resistant to one or more antibiotics, and not usually the cause of disease in the involved system, has become commonplace in patients treated with these agents. Such invasion has been most frequently observed in cases of superinfection of the urinary tract by members of the Pseudomonas and Proteus group. It is evident that the ever-widening use of antimicrobial agents will be associated with the discovery of infectious disease caused by a wide variety of unusual micro-organisms.

SUMMARY

Eleven cases of urinary-tract infection caused by a member of the Chromobacterium group, probably Serratia marcescens, are reported.

Bacteremia was present in two cases, in one of which a fatal endocarditis developed.

The bacteriological properties of the organism isolated are described.

All strains of the organism were highly resistant in vivo and in vitro to all antibiotics currently available.

It is postulated that the occurrence of this usually saprophytic bacterium in a pathogenic role may be, in part, the result of the increased use of antimicrobial agents.

BACTEREMIA DUE TO GRAM-NEGATIVE BACILLI OTHER THAN THE SALMONELLA

A Clinical and Therapeutic Study

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SEPSIS due to Escherichia coli was the subject of excellent reports by Jacob [1] in 1909 and Felty and Keefer [2] in 1924. However, the fact that strains and species of Gram-negative bacilli differ in their sensitivity to modern antimicrobial agents has made it desirable to study further not only bacteremias due to E. coli but those due to other non-lactose-fermenting Gram-negative rods.[3] The present study was undertaken to determine whether there was anything characteristic about the clinical pictures caused by different species of Gram-negative bacilli and to work out a therapeutic approach to patients in whom bacteremia due to these organisms was suspected. Presented in this report is a study of 29 cases of bacteremia due to Gram-negative bacilli other than Salmonella. Emphasis has been placed on the incidence of these infections, the clinical and laboratory picture caused by each type of bacilli, the sensitivities of the etiological strains to antibiotics, the possible role of antibiotics in allowing certain of the infections to be established, specific and nonspecific therapeutic measures, and the role of the laboratory in the management of these patients.

REVIEW OF THE LITERATURE

Brill and Libman [4] in 1899 were among the first to call attention to blood-stream invasion by Pseudomonas aeruginosa. The patient they described had probable cirrhosis of the liver, ran a downhill course, characterized by spiking fever, jaundice, pigmentation of the skin, and tonic convulsions, and died. In 1904 Eastman and Keene [5] reported a case of Bacillus pyocyaneus (Ps. aeruginosa) septicemia associ-

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1. Jacob, L.: Über Allgemeininfektion durch Bacterium coli commune, Deutsche Arch. klin. Med. **97**:303-347, 1909.

2. Felty, A. R., and Keefer, C. S.: Bacillus Coli Sepsis: Clinical Study of 28 Cases of Blood Stream Infection by Colon Bacillus, J. A. M. A. **82**:1430-1432 (May 3) 1924.

3. Waisbren, B. A., and Spink, W. W.: Comparative Action of Aureomycin, Chloromycetin, Neomycin, Q-19, and Polymyxin B Against Gram Negative Bacilli, Proc. Soc. Exper. Biol. & Med. **74**:35-40, 1950.

4. Brill, N. E., and Libman, E.: Pyocyaneus Bacillemia, Am. J. M. Sc. **118**:153-162, 1899.

5. Eastman, J. R., and Keene, T. V.: Bacillus Pyocyaneus Septicaemia Associated with Blastomycetic Growth in Primary Wound, Ann. Surg. **40**:613-632, 1904.

ated with blastomycosis. They emphasized the neurologic and psychic changes that accompanied the infection and mentioned the fact that in children petechiae form from which B. pyocyaneus can be cultured.

The excellent report of Jacob [1] on E. coli sepsis appeared in 1909. He reviewed 39 cases, of which 13 were his own. Sixteen of these patients died. The portals of entry were, in order of frequency, the biliary tract, the urinary tract, the gastrointestinal tract, and the female genital tract. Metastatic lesions were observed in 25% of the cases. A special point was made of the fact that a high leucocyte count was a bad prognostic sign. Felty and Keefer [2] in 1924 reported on 25 patients with sepsis due to E. coli, of whom eight died. The portals of entry, in their order of frequency, were the urinary tract, the female genital tract, the gastrointestinal tract, and wound infections. In none of their patients was the biliary tract the portal of entry. Operative manipulation preceded the blood-stream invasion in 19 patients. The clinical picture was not considered characteristic. Rigors were common. The temperature ranged from 100 to 107 F. The average duration of fever was nine days. Jaundice was present in three patients, and hemoglobinuria occurred in one patient, from whom a hemolytic colon bacillus was isolated. The leucocyte counts ranged from 3,600 to 38,000. In contradistinction to Jacob,[1] Felty and Keefer felt that a high leucocyte count was of favorable prognostic import. Metastatic lesions were noted in five cases. These were in the lungs and kidneys. The extent, severity, and location of the primary focus, rather than the sepsis itself, were believed to be of primary prognostic importance.

Other reports in the older literature in regard to E. coli sepsis were those of Holzman,[6] Panton and Tidy,[7] Pope,[8] and Draper.[9] The patients of Holzman [6] and Panton [7] all had clinical courses characterized by hard, shaking chills and fever. Pope's case, that of a 10-yr.-old boy who had a mild four-day illness characterized by general malaise, diarrhea, and backache, was of interest in that the organism was isolated from the blood. Recovery was uneventful. Multiple metastatic abscesses were the outstanding feature of the case of E. coli bacteremia reported by Draper.[9]

The more recent reports on E. coli sepsis were mostly concerned with sporadic cases of bacterial endocarditis.[10] The five cases of Phipps [10a] were all of debilitated

6. Holzman, A.: Two Cases of Colon Bacteraemia, Long Island M. J. **7**:376-378, 1913.

7. Panton, P. N., and Tidy, H. L.: A Note on the Occurrence of the Colon Bacillus in the Blood, Lancet **183**:1500, 1912.

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9. Draper, G.: A Case of Intrahepatic Biliary Calculi with Fatal Colon Bacillus Pyaemia, Bull. Ayre Clin. Lab. **10**:21-26, 1910.

10. (*a*) Phipps, C.: Acute Bacterial Endocarditis, New England J. M. **207**:768-770, 1932. (*b*) Hussey, H. H.; Keliher, T. F.; Schaefer, B., and Walsh, B. J.: Septicemia and Bacterial Endocarditis Resulting from Heroin Addiction, J. A. M. A. **126**:535-538 (Oct. 28) 1944. (*c*) Duhig, J. V.: Septicaemia and Acute Infective Endocarditis Due to Bacterium Coli, M. J. Australia **1**:434-437, 1933. (*d*) Harries, G. E., and Burtenshaw, J. M.: Acute Infective Endocarditis Due to Bacterium Coli, Lancet **2**:803-804, 1937. (*e*) Stevens, W. H., and Parks, J. L.: A Case of Acute Bacterial Endocarditis Caused by Escherichia Coli, J. Bact. **36**:606, 1938. (*f*) Fletcher, D. E.: Bacillus Coli Endocarditis, Am. Heart J. **34**:743-749, 1947. (*g*) Smith, A. G.: Endocarditis Due to Escherichia Coli, New England J. Med. **243**:129-132, 1950. (*h*) Hoffman, S. M.; Wellman, W. E., and Sayre, G. P.: Escherichia Coli Endocarditis: Report of a Case, Proc. Staff Meet., Mayo Clin. **26**:1-8, 1951.

patients who had fatal courses ranging from five to 14 days. Sulfadiazine therapy was ineffective in preventing the death of the patient reported on by Hussey and associates.[10b] Hypotension and a leucocyte count of 59,000 were the outstanding features of the case reported by Fletcher.[10f] The report of Hoffman, Wellman, and Sayre[10h] in 1951 reviewed the 13 well-documented cases of E. coli endocarditis in the literature. They found a surgical procedure to be the usual precipitating incident, and normal heart valves usually were involved. None of the patients was treated successfully. The increased incidence of bacteremia due to E. coli in cirrhotic patients was noted in 1894 by Hanot[11] and more recently by Whipple.[12]

Scott[13] in 1929 pointed out the frequency with which bacteria enter the blood stream as a result of urologic procedures. He made routine blood cultures on urologic patients, and in a two-year period obtained 82 positive cultures. The species of bacteria and mortality from each were as follows: E. coli, 33 cases, five deaths; Proteus, five cases, three deaths; Ps. aeruginosa, four cases, one death; Salmonella, five cases, no deaths; unclassified, 33 cases, two deaths. The clinical pictures and courses were not detailed in this account, but chills and fever appeared to be the usual response.[18]

Most of the recent reports of sepsis due to Ps. aeruginosa have concerned cases of bacterial endocarditis. The patient reported on by Kearns[14] had a six-week course, during which anemia developed, but there was no rise in leucocyte count. In 1937 Fish, Hand, and Keim[15] summarized the four cases of Pseudomonas endocarditis in the literature and added one of their own. Notable in their case was the lack of rise in the leucocyte count and the affinity of the organisms for the walls of blood vessels. Moragues and Anderson[16] in 1943 also noted this tendency for Pseudomonas to cause blood-vessel lesions. The review of Stanley[17] gave an excellent account of the modes of virulence of Ps. aeruginosa. Most of the cases of bacteremia that he noted occurred in debilitated patients. The severe prostration and the numerous petechiae of these patients were striking.

A dearth of clinical reports of bacteremia due to Aerobacter aerogenes was found in the literature. This is probably due to the fact that the early authors included this organism in the coli group. One case of A. aerogenes bacteremia was mentioned in a report from this laboratory,[18] and another was detailed by Duncan and co-workers.[19]

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11. Hanot, M. V.: Ictère grave hyperthermique sans coli-bacille, Compt. rend. Soc. biol. **46**:690-691, 1894.

12. Whipple, R. L.: B. Coli Septicemia in Laennec's Cirrhosis of the Liver, Ann. Int. Med. **33**:462-466, 1950.

13. Scott, W. W.: Blood Stream Infections in Urology: Report of 82 Cases, J. Urol. **21**:527-566, 1929.

14. Kearns, J. J.: Malignant Endocarditis Due to Bacillus Pyocyaneus, Arch. Path. **21**:839-843 (June) 1936.

15. Fish, G. W.; Hand, M. W., and Keim, W. F.: Acute Bacterial Endocarditis Due to Pseudomonas Aeruginosa, Am. J. Path. **31**:121-127, 1937.

16. Moragues, V., and Anderson, W. A. D.: Endocarditis Due to Pseudomonas Aeruginosa, Ann. Int. Med. **19**:146-154, 1943.

17. Stanley, M. M.: Bacillus Pyocyaneus Infections, Am. J. Med. **2**:253-277; 347-367, 1947.

18. Waisbren, B. A., and Spink, W. W.: A Clinical Appraisal of Neomycin, Ann. Int. Med. **33**:1099-1119, 1950.

19. Duncan, G. G.; Clancy, C. F.; Wolgmot, J. R., and Beidleman, B.: Neomycin: Results of Clinical Use in 10 Cases, J. A. M. A. **145**:75-80 (Jan. 13) 1951.

Agents used in the treatment of infections due to Gram-negative bacilli prior to the sulfonamide era are now of only historical interest. They included vaccine therapy, and intravenous administration of merbromin (mercurochrome®), colloidal manganese, and 1% acetic acid.[20] The patient with E. coli bacteremia reported on by Whipple [12] showed a questionable response to treatment with the combination of streptomycin and sulfadiazine. Collins and associates [21] reported a doubtful response to aureomycin therapy in a case of E. coli bacteremia. Duncan and colleagues [19] successfully treated a patient who had A. aerogenes bacteremia with neomycin. Stanley [17] felt that a case of bacteremia due to Ps. aeruginosa responded clinically to streptomycin therapy in spite of an eventually fatal outcome.

MATERIALS AND METHODS

The 29 cases of bacteremia due to Gram-negative bacilli, the analysis of which constitutes the body of this report, all were observed by me in the wards of either the Minneapolis General Hospital or the University of Minnesota Hospital. In each of the cases included there was no reasonable doubt that the organism isolated from the blood stream was of clinical significance. The strains of E. coli, A. aerogenes, Ps. aeruginosa, Proteus, and Alcaligenes were identified by the bacteriology laboratories of the two hospitals. The strain of Eberthella oedematiens and the unclassified bacillus were studied by Dr. Henry Bauer, of the Minnesota State Board of Health.

The sensitivities of the bacteria to various antibiotics were determined by a tube-dilution method. Five-tenths-milliliter aliquots of a 1:100 dilution of an 18-hr. growth in dextrose broth were added to tubes (13 by 100 mm.) containing 0.5 ml. of decreasing concentrations of drug. The end-point was determined by selecting the tube with the lowest concentration of antibiotic in which visual growth of the organism was inhibited for 18 hr. When combinations of antibiotics were tested, 0.5 ml. of different concentrations of each drug was placed in the tubes and enough of the 1:100 dilution of the organism in broth was added to keep the 1:1 ratio of 0.9% sodium chloride solution and broth.

An analysis of this method was made and will be reported elsewhere.[22] It showed that the method was reasonably accurate, that dextrose broth did not inhibit any of the antibiotics, and that strains isolated from an infection at a single time were representative of the majority of the organisms causing the infection.

CLINICAL AND LABORATORY FINDINGS

E. Coli.—Ten patients with bacteremia due to E. coli were studied (Table 1). The positive cultures from nine of them were obtained at the Minneapolis General Hospital and represent 0.64% of all blood cultures taken in a nine-month period and 21% of all those that were positive (Table 2). Seven cases were of women and three of men. The age range was 22 to 70 yr. Only one of the patients had cirrhosis prior to infection. There were two deaths; one was due to a large perinephritic abscess and the other to multiple peritoneal abscesses. The portals of entry were as follows: genitourinary tract, six cases; uterus, one case; gall bladder or bile ducts, two cases; peritoneal cavity, one case (Table 3).

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20. Hussey and others.[10b] Duhig.[10c] Dudgeon, E. S.: Bacterial Vaccines and Their Position in Therapeutics, New York, Paul B. Hoeber, Inc., 1927.

21. Collins, H. S.; Paine, T. F., Jr.; Wells, E. B., and Finland, M.: Aureomycin, a New Antibiotic: Evaluation of Its Effect in Typhoid Fever, Severe Salmonella Infections and in a Case of Colon Bacillus Bacteremia, Ann. Int. Med. **29**:1077-1092, 1948.

22. Waisbren, B. A.; Carr, C., and Dunnette, J.: The Tube Dilution Method of Determining Bacterial Sensitivity to Antibiotics, Am. J. Clin. Path., to be published.

TABLE 1.—*Cases of Bacteremia Due to Gram-Negative Bacilli Other Than Salmonella*

Organism	No. of Cases	No. of Patients		Age Range, Yr.	Deaths
		F	M		
E. coli	10	7	3	22-70	2
A. aerogenes	7	1	6	25-75	0
Ps. aeruginosa	5	2	3	3-79	0
Proteus	5	0	5	48-93	2
Alcaligenes	1	..	1	51	0
Paracolon	1	..	1	76	0
Eberthella oedematiens	1	..	1	74	1
Unclassified	1	..	..	3	0
Totals	31*	10	21	3-93	5

* Twenty-nine patients were observed, but two of these had two separate episodes of bacteremia due to different Gram-negative bacilli.

TABLE 2.—*Organisms Isolated from 1,396 Blood Cultures Drawn from July 1, 1950, to April 1, 1951, at the Minneapolis General Hospital.**

Organism	No. of Patients	% of Positive Cultures	% of Total
Pneumococci	12	27.5	0.87
Group No. Group No. A 4 D 1 B 3 E 1 C 2 Not grouped 1			
Staph. pyogenes var. aureus (coagulase positive)	2	4.5	0.14
Staph. pyogenes var. albus (coagulase positive)	2	4.5	0.14
Enterococci	3	6.8	0.22
E. coli	9	21.0	0.64
A. aerogenes	5	11.0	0.30
Ps. aeruginosa	2	4.5	0.14
Proteus	2	4.5	0.14
Alcaligenes	2	4.5	0.14
Neisseria meningitidis	2	4.5	0.14
Unclassified	1	2.2	0.07
Eberthella oedematiens	1	2.2	0.07
Salmonella typhimurium	1	2.2	0.07
Totals	44	99.9	3.4

* Mixed cultures and obvious contaminants were omitted from this tabulation and subtracted from the total. The 1,396 cultures were drawn from 980 patients.

TABLE 3.—*Portal of Entry of Gram-Negative Bacilli That Caused Bacteremia*

Organism	Unknown	Genito-urinary Tract	Gastro-intestinal Tract	Uterus	Gall Bladder or Bile Ducts	Peri-toneum
E. coli	..	6	..	1	2	1
A. aerogenes	1	6	..	..	..	..
Ps. aeruginosa	..	4	..	..	..	1
Proteus	..	5	..	..	..	..
Alcaligenes	..	1	..	..	..	..
Paracolon	..	1	..	..	..	..
Eberthella oedematiens	..	..	1	..	..	..
Unclassified	..	1	..	..	..	..
Totals	1	24	1	1	2	2

Two distinct clinical pictures were presented by these patients (Table 4). One group appeared hot, dry, and flushed. They were animated and alert, and their movements were short and jerky. Their rectal temperatures ranged from 102 to 106 F. Their pulses were full and bounding, and their blood pressures remained at the level they had been previous to the onset of the bacteremia. These patients did not give one the impression of being gravely ill. They were arbitrarily classified as toxic, as compared with a group of patients who presented a shock-like picture. This group was comprised of three patients. They all appeared critically ill and, in spite of high temperature responses, were cold, clammy, and lethargic. Their blood pressures fell rapidly and did not return to original levels until the infection had subsided.

The laboratory findings were not markedly different in the two groups. Although the urine output decreased greatly in two of the three patients who went into shock, it decreased similarly in two patients who appeared toxic. In five of the patients enough data were obtained to determine whether or not hemolysis occurred as a result of the bacteremia. In only one case (C7) [23] was evidence of hemolysis

TABLE 4.—*Clinical Picture Caused by Bacteremia Due to Gram-Negative Bacilli*

Organism	Relatively Asymptomatic	Toxic	Shock-like
E. coli	..	7	3
A. aerogenes	1	3	2
Ps. aeruginosa	3	1*	2
Proteus	1*	..	5
Alcaligenes	..	1	..
Paracolon	..	..	1
Eberthella oedematiens	..	..	1
Unclassified	..	1*	1

* These patients evidenced more than one clinical picture at times that blood cultures yielded organisms.

obtained. It is of interest that the strain of E. coli isolated from this patient was not hemolytic when streaked across a blood-agar plate.

An increase in the total and relative number of polymorphonuclear leucocytes was noted in the majority of cases. It is of possible significance that two of the three patients who presented the shock-like picture had relatively low white blood cell counts, with a high percentage of polymorphonuclear leucocytes. As soon as one of these patients (C7) was brought out of shock, his leucocyte count rose to 50,000, with 95% polymorphonuclear leucocytes.

Blood chemical values determined at the time of the bacteremia, for the most part, were not striking. In the one case in which marked nitrogen retention developed (C6), bilateral renal calculi and hydronephrosis were present. A rise in blood sugar was noted in two patients who were not diabetic (C5, C6). While this probably represents a so-called starvation diabetes, one might speculate that it was caused by a reaction to the toxin of E. coli similar to that displayed by rabbits.[24]

The patients studied form too small a group from which to draw definite conclusions regarding the mortality rate and natural course of E. coli bacteremia, but the

23. Code for case numbers is explained in legend to figure.

24. Zeckner, I. T., and Goodell, H.: Rapid Alterations of Blood Sugar Levels of Rabbits as a Result of Intravenous Injections of Killed Bacteria of Various Types, J. Exper. Med. **42**:43-56, 1925.

variety of responses encountered in the six patients who either received no specific therapy or received apparently ineffective chemotherapy is worth noting. Of the two patients that died, one (C6) had a 12-day septic course with gradual weakening and death, and the second (C8) went into shock and died in three hours. The other four patients were acutely ill for one to seven days and made uneventful recoveries. One variable that might explain these differences is the number of E. coli that invaded the blood stream in each case. Unfortunately, pour plates were not obtained from enough of the patients to determine the correlation between the number of bacteria and the natural course of the infection.

A. Aerogenes.—Seven patients with bacteremia due to A. aerogenes were studied (Table 1). The positive cultures of five were obtained at the Minneapolis General Hospital and represent 0.3% of all blood cultures taken during a nine-month period and 11% of all those that were positive (Table 2). One patient was a woman and the seven others were men. The age range was 25 to 75 years. There were no fatalities. One of the patients probably had cirrhosis. The portal of entry was not clear in one case and was the genitourinary tract in the remaining six (Table 3).

Both the toxic and the shock-like clinical pictures seen in patients with E. coli bacteremia also were seen among these patients (Table 4). In addition, two persons (A3, A4) were essentially asymptomatic at the time A. aerogenes organisms were circulating in the blood stream (Table 4).

The laboratory findings in this group of patients were similar to those in patients infected with E. coli. Hemolysis was noted in only one instance. The temperature response was greater than 102 F., with but one exception. The blood pressures of the two patients with the shock-like picture fell markedly, but, interestingly, their urine output remained adequate. On the other hand, one of the asymptomatic patients (A3) had a pronounced fall in blood pressure and urinary output. The two patients who presented the shock-like picture each had leucocyte counts greater than 20,000. In this way they differed from the similar group among the patients with E. coli bacteremia, who had low leucocyte counts. The blood chemical values of one patient (A2) are of interest, in that they showed development of nitrogen retention in the face of a normal output. His blood urea nitrogen rose from 19 to 73 mg. per 100 ml. during the acute phase and returned to 24 mg. per 100 ml. when the infection was controlled.

Three of the seven patients (A1, A3, A6) were treated ineffectively or belatedly enough to reveal information concerning the natural history of this infection. One of these (A1) is of particular interest in that she apparently had been suffering from intermittent bacteremia due to A. aerogenes for a five-year period without any apparent permanent harm.[18] The other two patients (A3, A6), both treated with antibiotics to which organisms from their blood stream were resistant, recovered gradually over a period of a week to 10 days.

Ps. Aeruginosa.—There were five patients with bacteremia due to Ps. aeruginosa (Table 1). Cultures from two of them were obtained at the Minneapolis General Hospital and represent an incidence of 0.14% of all cultures and of 4.5% of those that were positive (Table 2). Two patients were female and three were male, and the age range was 3 to 79 yr. The portal of entry was the genitourinary tract in four cases and the peritoneal cavity in the fifth (Table 3). There were no fatalities.

The outstanding clinical feature in three of these cases (Ps1, Ps2, Ps3) was the apparent well-being with which the patients tolerated the organisms in their blood stream (Table 4). Although they did have fever, they had no complaints, appeared clinically well at the time the blood cultures were drawn, and suffered no drop in blood pressure. The other two patients exhibited the reaction I have designated as shock-like, had a marked fall in blood pressure, and showed definite evidence of hemolysis. The three patients in whom counts were made had an absolute and relative increase in polymorphonuclear leucocytes. Changes in the blood chemical values of these patients were not striking.

The two patients in whom specific chemotherapy was apparently ineffective (Ps1, Ps2) had approximately a five-day course of elevated temperature and made uneventful recoveries.

Proteus.—Five male patients with bacteremia due to Proteus were observed (Table 1). The positive cultures for two of the patients were obtained at the Minneapolis General Hospital and represent 0.14% of all cultures and 4.5% of those that were positive (Table 2). The age range was 48 to 93 yr. One of the two patients who died was 59 years old and the other was 62 years of age. Cirrhosis was present in one patient. The genitourinary tract was the probable portal of entry in all five cases (Table 3).

The striking feature in this small group of cases of Proteus bacteremia was the catastrophic nature of the illness. All five patients presented a shock-like picture and appeared moribund. It was thought that without active and specific intervention all would have died. On the other hand, on one occasion Proteus was isolated from the blood of the 93-year-old patient (Pr3) at a time that he was relatively asymptomatic. Ten days later, when Proteus was again isolated from this same patient, he was in deep shock.

The temperature responses were not unlike those in the patients with bacteremias due to other Gram-negative bacilli. There was a marked fall in blood pressure in all the patients, but three of them were able to maintain a normal urine output. Hemolysis was noted in two instances. Neither the total leucocyte counts nor the percentages of polymorphonuclear leucocytes were as great in this group of patients as in those infected with the other species. This suggests that Proteus does not evoke as great a polymorphonuclear-leucocyte response as do E. coli, Ps. aeruginosa, and A. aerogenes.

Four of the five patients suffered nitrogen retention during the acute phase of their illness. Two of these persons had maintained an adequate urine output during the time their nitrogen retention was rising. The two patients who died exhibited a marked rise in blood urea nitrogen, and in one (Pr1) uremia was the direct cause of death.

Other Gram-Negative Bacilli.—Alcaligenes, Paracolon, Eberthella oedematiens,[25] and an unclassified organism[26] were the other Gram-negative bacilli isolated from

25. This organism fitted exactly the criteria for Eberthella oedematiens as outlined by Bergey, D. H.: Bergey's Manual of Determinative Bacteriology, ed 3, Baltimore, Williams & Wilkins Company, 1930. In later editions of the Manual this species has been omitted.

26. This was a Gram-negative coccic bacillus. Limited determinations indicated that this organism may fit best into the Achromobacter group. For convenience, in the remainder of this paper this organism will be referred to as the "unclassified bacillus."

the patients described in this report. This group of patients as a whole showed about the same types of responses as were seen in the group whose illness was due to the commoner Gram-negative bacilli. Both toxic and shock-like clinical pictures were seen (Table 3), and the laboratory data showed about the same range as encountered in the other patients. Since the Eberthella oedematiens and the species into which the unclassified coccic bacilli could fall never before have been reported as pathogenic, the severe diseases they caused are worthy of special note (Ps4U1, S1). In both of these cases there can be no doubt that the organisms were in fact virulent for the persons concerned, since in one case (Ps4U1) five positive cultures were obtained and in the other (S1) the Eberthella oedematiens was isolated from the blood and spinal fluid before death and from peritoneal exudate after death.

Summary of Clinical Picture and Laboratory Findings.—Twenty-two of the 29 patients were observed at the Minneapolis General Hospital. Their cultures represented 3.4% of all blood cultures drawn in nine months and 55% of those that were positive. The portal of entry was the genitourinary tract in 24 cases, the biliary tract in two cases, the peritoneal cavity in two cases, and the uterus and the gastrointestinal tract each in one case (Table 3).

Three distinct clinical pictures were presented by these patients. They were asymptomatic, toxic, or in a shock-like state at the time the positive blood cultures were drawn. The 13 patients classified as toxic appeared hot, dry, flushed, and animated, while the 15 patients described as being in a shock-like state were cold, clammy, apprehensive, hypotensive, and lethargic. Four of the five patients who died and all the patients with bacteremias due to Proteus were in the latter group. A temperature response greater than 102 F. was exhibited by all but one of the patients. The range was 102 to 107 F. Fifteen of the patients had a marked fall in urinary output, but, interestingly, five of the patients in the shock-like group were able to maintain adequate urinary function. Hemolysis occurred during the bacteremia of eight of the patients. This was not always correlated with in vitro hemolytic activity of the organisms.

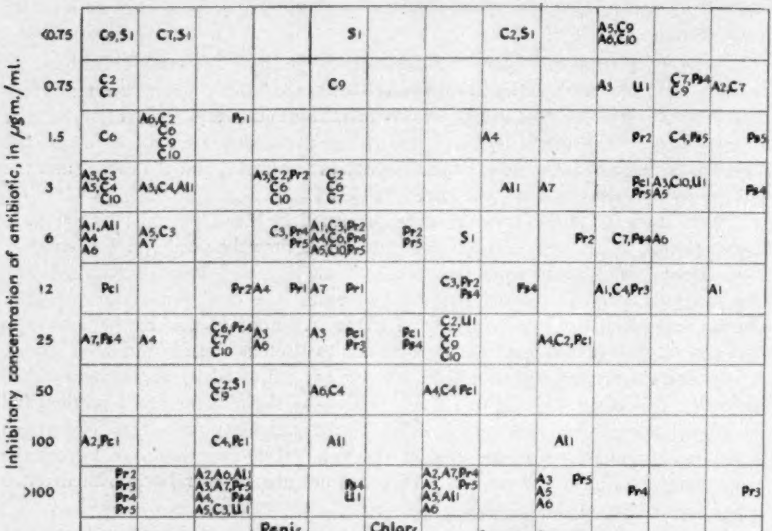
A relative and absolute increase in the number of polymorphonuclear leucocytes was noted in the majority of the cases. This was less remarkable in the cases of Proteus bacteremia than in those due to the other organisms. Eleven patients had significant increases in the concentration of blood urea nitrogen during the acute phase of the disease. It was reversible in the patients who recovered, but in one patient (Pr1) death was due to continued renal failure and the development of uremia.

THERAPY

The discussion on each of the antibiotics that follows is offered with the assumption that, even though the benefit of chemotherapy for these infections has not been statistically proved, all patients for whom the diagnosis of bacteremia due to Gram-negative bacilli is entertained will receive antibiotic therapy. The data derived from study of each of the 29 patients were analyzed for information that would be of help in guiding this therapy (Figure). Particular attention was paid to the in vitro synergistic or antagonistic activity of combinations of antibiotics and its relation to clinical results. The possible influence of antibiotics in establishing specific cases of blood-stream invasion was studied by determining the drugs the

patients were receiving at the time of their positive blood cultures (Table 5). In addition, the tube-dilution method of determining bacterial sensitivity to antibiotics was assessed in regard to both its usefulness in the management of these patients and the correlation between in vitro results derived from it and the clinical course of the patients.

Penicillin.—Thirteen of the 29 patients were receiving penicillin at the time a Gram-negative bacillus was isolated from their blood. This demonstrated the ineffectiveness of this drug as a prophylactic agent against Gram-negative infections (Table 5). On the other hand, 11 of the strains were inhibited in vitro by 60



Comparative sensitivities to antibiotics and combinations of antibiotics of strains of Gram-negative bacilli isolated from the blood.

The tube-dilution method of determining bacterial sensitivity to antibiotics was used throughout.

Letters refer to case numbers as in text, i. e., C9 represents Case 9 of E. coli bacteremia. *C*=E. coli. *A*=A. aerogenes. *Ps.*=Ps. aeruginosa. *Pr.*=Proteus. *S*=Eberthella oedematiens. *U*=Unclassified bacilli. *Pc*=Paracolon. *Al*=Alcaligenes.

The values for penicillin represent concentrations in units per milliliter (1 unit=0.6μ).

units of penicillin or less (Figure). This is a concentration obtainable by the intravenous injection of 0.6 gm. (1,000,000 units) of penicillin.[27] In three of the cases (C7, Pr1, Pr2) the evidence suggested that penicillin favorably affected the course of the infection. The fact that these favorable results were not observed in cases in which large doses of penicillin were used against resistant organisms

27. Unpublished data from our laboratory.

suggested that they were due to the action of the drug against the sensitive bacteria rather than to the antitoxin activity of penicillin.[28]

Worthy of particular note is the sensitivity of many strains of Proteus (Pr1, Pr2, Pr4) to the action of penicillin (Figure). Since aureomycin, chloramphenicol, streptomycin, and polymyxin are ineffective against this species, it is particularly helpful to be able to add penicillin to chloramphenicol as an antibiotic combination that can be used with a fair chance of success against Proteus infections.[3]

Aureomycin.—Eight of the patients were receiving aureomycin at the time of the positive blood culture. Three of the five patients with Proteus bacteremia were among these eight persons (Table 5).

In vitro aureomycin was the drug to which 15 of the strains were most sensitive. This is of particular interest when one considers that aureomycin is the most unstable of the antibiotics and loses more than 90% of its activity by the time the test reaction is read.[22] Thus, if all other factors were equal, aureomycin would be most unfavorably compared by this in vitro determination. This suggests that the actual superiority of this drug is even greater than that indicated by the

TABLE 5.—*Antibiotics Patients Were Receiving at the Time Gram-Negative Bacilli Were Isolated from Blood*

Organism	None	Penicillin	Strepto-mycin	Aureo-mycin	Chloram-phenicol	Sulfa-diazine	Q-19
E. coli	7	2	1	1	..	..	..
A. Aerogenes	1	5	2	..	..	2	..
Ps. aeruginosa	1	2	2	1	1	..	2
Proteus	2	3	1	3	1	1	..
Alcaligenes	..	1	..	..	..	1	..
Paracolon	..	..	..	1	..	..	..
Eberthella oedematiens	..	..	..	..	..	..	..
Unclassified bacillus	..	..	1	..	1	..	..

comparative tube-dilution test. On the basis of this information and the clinical results, it is apparent that aureomycin is the drug of choice for treatment of the bacteremia due to the coliform organisms (Fig.). The two cases of Pseudomonas bacteremia that responded well to aureomycin therapy indicate that this drug also is useful in some cases of bacteremia due to this organism. However, three strains of the coliform group and one of Pseudomonas were isolated while the patients were receiving aureomycin (Table 5). This indicates that one will not be safe in assuming that all cases of bacteremia due to these organisms will respond to therapy with aureomycin.

Chloramphenicol.—Three of the patients were receiving chloramphenicol at the time their positive blood cultures were drawn (Table 5). The preeminence of chloramphenicol for the treatment of Proteus infection is illustrated by the fact that the strains isolated from four of the five patients with Proteus bacteremia were the most sensitive to this antibiotic (Fig.). This confirms previous work in which chloramphenicol was shown to be the best of the available antibiotics against Proteus.[3] The strain isolated from the fifth patient was also sensitive to chloramphenicol (Pr1) but more sensitive to penicillin. Since the blood concentrations

28. Boor, A. K., and Miller, C. P.: Effect of Penicillin on the Lethal Action of Meningococcal Endotoxin in Experimental Animals, Science **102**:427-428, 1945.

obtained with chloramphenicol are approximately 10 times as high as those obtained with comparable doses of aureomycin, it is possible that organisms with relatively high in vitro resistance might respond to the clinical use of this drug.[29] Viewed in this light, chloramphenicol may be an acceptable drug for treatment of bacteremias due to the coliform group, as well as those due to Proteus. Reports of the clinical effectiveness of this drug in urinary-tract infections add credence to this possibility.[30]

Streptomycin.—Seven of the patients were receiving streptomycin at the time cultures of their blood yielded Gram-negative bacilli (Table 5). One of the strains of Ps. aeruginosa was most sensitive to this antibiotic (Fig.). However, the existence of streptomycin-resistant variants in every strain and the rapidity with which sensitive strains become resistant as a result of this mitigate against the use of streptomycin alone for any bacterial infection (with the possible exception of some types of tuberculosis).[31] On the other hand, streptomycin has a potentiating effect on some of the other antibiotics and undoubtedly has a place in the treatment of many infections when used in combination with other agents.[32] As will be discussed below, it apparently had a potentiating effect that was of clinical importance in at least three of the cases in this series (Pr2, A6, C3).

Sulfadiazine.—Four of the patients were receiving sulfadiazine at the time of their positive blood cultures (Table 5). Nine patients received sulfadiazine at one or another time during their course, and in no instance was there any evidence that it was of benefit. While it cannot be denied that sulfadiazine is effective in treatment of certain infections due to the coliform group, there are more effective agents available that should be used in therapy of bacteremias caused by coliform organisms. Unfortunately, the inhibiting action of most culture mediums against sulfadiazine makes it impossible to use the standard tube-dilution test to compare its in vitro activity with that of the antibiotics.[33]

Neomycin.—In only two cases was it necessary to use this ototoxic and nephrotoxic agent.[18] In the first case neomycin was used before its toxicity was established and in the second when in vitro tests revealed it to be by far the most active agent against the unclassified bacillus isolated from the blood of the critically ill child. Although in these cases use of the drug was in all probability lifesaving, the fact that the child became deaf after its administration reemphasizes the fact that this antibiotic is too toxic for general use.[18] This is unfortunate because, as was found previously, most of the species of Gram-negative bacilli are susceptible to its activity (Fig.).[3]

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29. Ley, H. L.; Smadel, J. E., and Crocker, T. T.: Administration of Chloromycetin to Human Subjects, Proc. Soc. Exper. Biol. & Med. **68**:9-12, 1948.

30. Garvey, F. K.; Cline, W. A., and Meads, M.: Chloramphenicol in Bacillary Infections of the Urinary Tract, South. M. J. **43**:85-90, 1950.

31. Demerec, M.: Patterns of Bacterial Resistance to Penicillin, Aureomycin and Streptomycin, J. Clin. Invest. **28**(Pt. 2):890-893, 1949.

32. (*a*) Robbins, C., and Tompsett, R.: The Summation of Penicillin and Streptomycin Activity in Vitro and in the Treatment of Subacute Bacterial Endocarditis, J. Clin. Invest. **28**:1043-1044, 1949. (*b*) Jawetz, E.; Gunnison, J. B., and Coleman, V. B.: The Combined Action of Penicillin with Streptomycin or Chloromycetin on Enterococci in Vitro, Science **111**:254-256, 1950.

33. Spink, W. W., and Vivino, J. J.: Sulfonamide-Resistant Staphylococci: Correlation of in Vitro Sulfonamide Resistance with Sulfonamide Therapy, J. Clin. Invest. **23**:267-278, 1944.

Q-19 [34].—This report is the first in which the clinical use of this antibiotic has been detailed. Q-19, discovered in 1948 by Murray and Tetrault,[35] is a polypeptide and is related to the polymyxin-aerosporin type of antibiotic. Studies of its clinical use and in vitro activity have been going on in this laboratory for the past two years.[3] It has been administered parenterally to 30 patients without any serious toxic effects. Although it is the most effective antibiotic against Ps. aeruginosa in vitro, the dosage of Q-19 used did not have an appreciable effect in two cases of Pseudomonas bacteremia. On the other hand, Q-19 undoubtedly saved the life of a patient (A2) who was suffering from A. aerogenes bacteremia. The apparent lack of toxicity of Q-19 and its in vitro activity certainly warrant further investigation of this agent.

Combinations of Antibiotics.—Recent reports have mentioned adverse clinical effects as possibly resulting from the use of combinations of antibiotics.[36] These suggestions have been based on evidences of in vitro antagonisms which occurred when bacteriostatic and bactericidal antibiotic agents were mixed. The explanation was advanced that the antagonisms occurred when the bacteriostatic agents prevented the bacteria from multiplying and thus becoming susceptible to the bactericidal agents [36b] (penicillin, for instance, kills only multiplying bacteria). Specifically the addition of aureomycin, chloramphenicol, or terramycin to penicillin has been shown to decrease the rate of penicillin's bactericidal activity.[37] However, in the same papers in which this was demonstrated, it was also shown that bacteriostatic synergism occurred when these agents were combined.[36b] Whether or not these drugs are used in combination should then depend on whether it is the rate of bactericidal activity or the bacteriostatic effect that determines the efficacy of an antibiotic. This question will be answered only by controlled clinical studies of patients to whom combinations of antibiotics are administered. However, the efficacy of primarily bacteriostatic agents, such as aureomycin and chloramphenicol, leads me to feel that in vitro tests that show a high degree of bacteriostatic activity will correlate better with satisfactory clinical results against acute infections than will tests showing rapid bactericidal activity with less inhibition. The recent report of Spies and co-workers [38] of clinical synergism between aureomycin and penicillin lends credence to this view.

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34. Q-19 has been referred to as circulin. It is a different antibiotic from the one described by McLeod (J. Bact. **56**:749-754, 1948; see also Garson, W., and others: J. Bact. **58**:115-116, 1949), which was originally named circulin but has since been renamed polypeptin. The Q-19 used in this study was obtained from The Upjohn Company, Kalamazoo 99, Mich.

35. Murray, F. J.; Tetrault, P. A.; Kaufmann, O. W.; Koffler, H.; Peterson, P. H., and Colingsworth, D. R.: Circulin: An Antibiotic from an Organism Resembling Bacillus Circulans, J. Bact. **57**:305-312, 1949.

36. (*a*) Jawetz and others.[32b] (*b*) Gunnison, J. B.; Jawetz, E., and Coleman, V.: Effects of Combinations of Antibiotics on Enterococci in Vitro, J. Lab. & Clin. Med. **36**:900-911, 1950. (*c*) Jawetz, E., and Speck, R. S.: Joint Action of Penicillin with Chloramphenicol on an Experimental Streptococcal Infection in Mice, Proc. Soc. Exper. Biol. & Med. **74**:93-96, 1950. (*d*) Gunnison, J. B.; Coleman, V. R., and Jawetz, E.: Interference of Aureomycin and of Terramycin with Action of Penicillin, ibid. **75**:549-552, 1950. (*e*) Keefer, C. S.: Antibiotics: Yesterday, Today, Tomorrow, Ann. Int. Med. **32**:582-589, 1950.

37. Jawetz and others.[32b] Gunnison and others.[36d]

38. Spies, H. W.; Dowling, H. F.; Lepper, M. H.; Wolfe, C. K., and Caldwell, E. R., Jr.: Aureomycin in the Treatment of Bacterial Endocarditis: Report of Nine Cases Together with a Study of the Synergistic Action of Aureomycin and Penicillin in One Case, A. M. A. Arch. Int. Med. **87**:66-78 (Jan.) 1951.

Among the cases in this report there were two in which in vitro bacteriostatic [39] synergism apparently correlated with clinical results (Pr2, Ps4U1). In one case penicillin and chloramphenicol were synergistic against Proteus, and in the other neomycin and aureomycin were synergistic against the unclassified bacillus. The former case prompted a further investigation of the action of chloramphenicol and penicillin against Proteus. It was found that not infrequently these two drugs are synergistic against this organism both in vitro and clinically.[40]

Twenty of these patients received combinations of antibiotics, and in no instance was significant in vitro or clinical antagonism noted (Fig.). In addition, no published reports of clinical antibiotic antagonism were found. Although there are obvious reasons why combinations of antibiotics should not be given if one alone will suffice, the evidence seems to indicate that in doubtful acute cases more good than harm is likely to result from the use of combinations of antibiotics.

Nonspecific Therapy.—The small amount of space devoted to the nonspecific measures given these patients should serve to deemphasize their importance. The patients seen in a shock-like state undoubtedly received almost as much benefit from measures used to restore effective circulation as they did from antibiotic therapy. Blood and plasma transfusions, elevation of the foot of the bed by shock blocks, administration of oxygen, infusions containing electrolytes and sugar, phenylephrine (neo-synephrine®) hydrochloride, and procaine hydrochloride intravenously, and use of tepid sponges were some of the measures employed that proved of value.

There were no cases in this series that suggested that the antibiotics had an antitoxic as well as an antibacterial activity. The reports of antitoxic activity of aureomycin, penicillin, chloramphenicol, terramycin, and sulfadiazine suggested these antibiotics might have been of value against toxins of bacteria that they did not inhibit.[41] However, in each case in which a specific therapeutic response resulted, the organism was sensitive in vitro to the antibiotic concerned. Therefore, since the antimicrobial agents do not appear to affect the toxemia accompanying bacteremia, one must use every effort at one's disposal to accomplish this by other means.

Use of Tube-Dilution Sensitivity Test.—This study established that in certain cases in vitro sensitivity tests can be of help in the clinical management of the patient. In Case Pr2, for instance, a prolonged clinical response was obtained only after the combination of the three drugs shown to be the most bacteriostatic in vitro was administered. In Case Ps4U1, the patient was pursuing a steadily downhill course until treatment with a combination of neomycin and aureomycin, shown to be synergistic in vitro, was started. When certain drugs that Patient Pr5 was receiving were shown to be ineffective in vitro their use was discontinued with a resultant saving of expense and discomfort to the patient.

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39. The tube-dilution test measures bacteriostatic activity.

40. Waisbren, B. A., and Carr, C.: Penicillin and Chloramphenicol in the Treatment of Infections Due to Proteus Organisms, to be published.

41. Boor and Miller.[28] Quan, S. F.; Chen, T. H., and Meyer, K. F.: Protective Action of Antibiotics Against Toxin of Pasteurella Pestis in Mice, Proc. Soc. Exper. Biol. & Med. **75**:548-549, 1950. Carpenter, C. M.; Hawley, P. L., and Barbour, G. M.: Protective Effects of Sulfanilamide Against Gonococcal Toxin, Science **88**:530-531, 1938.

A thorough knowledge of the bacterial spectrum of each antibiotic will be sufficient for one to make the correct choice of antibiotic in most cases. However, certain strains of each species do not follow the sensitivities to antibiotics of the majority (Fig.). It is in these cases that in vitro sensitivity tests are of particular value.

Summary of Therapy.—The number of patients treated in this study was too small to prove the value of any particular agent in the treatment of patients with bacteremia due to Gram-negative organisms. However, a careful study of individual cases revealed information that was helpful in developing a therapeutic approach to this condition. When confronted with a patient for whose disorder this diagnosis is entertained, it is of importance to determine which antimicrobial agents he has been receiving. Rather than increasing the dose of these agents, a new therapeutic regimen should be started. If the patient had not been receiving chemotherapy, the chances are best that the organism is of the coliform group and that it will respond readily to aureomycin therapy. If the patient has been receiving aureomycin, he may well have a Proteus bacteremia, which has the best chance of response to penicillin and chloramphenicol therapy. If the patient is critically ill, the three agents should be given simultaneously until a specific bacteriologic diagnosis is available. All organisms isolated from the blood should be saved, and, if a prompt clinical response is not forthcoming, a tube-dilution sensitivity test using individual antibiotics and combinations should be performed. The combinations of antibiotics likely to be synergistic are penicillin and aureomycin, penicillin and streptomycin, penicillin and chloramphenicol, and aureomycin and streptomycin.[42] In cases that are particularly difficult the tube-dilution test may be used to determine whether three antibiotics used in combination might not be of help.

In addition, nonspecific measures should be vigorously used in the symptomatic treatment of the patient.

COMMENT

Although the extent of the impact of antimicrobial therapy on the bacterial world is not known, evidence is accumulating that widespread changes in regard to the number of drug-resistant organisms have occurred.[43] Whether or not these changes among bacteria will start chain reactions that result in hastened evolution of nonpathogens to pathogens remains to be seen. The fact that two of the cases in this report (S1, U1) were due to organisms never before considered virulent may indicate that this possibility is not entirely a remote one.

Since three of the five patients with severe Proteus bacteremia were receiving aureomycin, one might speculate that if it were not for the suppressive effect of aureomycin on the coliform bacilli the Proteus might never have had the opportunity to get established in the blood stream. Thus, if aureomycin had not been given, these patients may have had only E. coli bacteremia, which appears to be milder than that due to Proteus. Viewed in this light, the Proteus bacteremias were indeed iatrogenic. To what extent, then, should prophylactic antibiotics be used if they

42. Jawetz and others.[32b] Spies and others.[38]

43. (*a*) Garrod, L. P.: The Reactions of Bacteria to Chemotherapeutic Agents, Brit. M. J. **1**:205-210, 1951. (*b*) Spink, W. W.: Clinical and Biologic Significance of Penicillin Resistant Staphylococci, Including Observations with Streptomycin, Aureomycin, Chloramphenicol, and Terramycin, J. Lab. & Clin. Med. **32**:278-293, 1951.

may result in the establishment in the blood stream of pathogenic organisms which usually are controlled by the normal bacterial flora of the body?

The cases presented in this report emphasize, as did Felty and Keefer,[2] the importance of instrumentation as a provocative cause of blood-stream invasion. Nineteen of the 29 patients had either operations or catheterizations that probably precipitated the bacteremia. Cirrhosis as a predisposing factor was not outstanding in this series, since only four of the patients had evidence of liver disease. This represents an incidence of liver disease that would be expected in a city-hospital population. The genitourinary tract was the portal of entry in the majority of these cases, as it was in the series of Felty and Keefer.[2]

The two distinct clinical pictures seen in these patients do not seem to have been emphasized previously in the literature. The type of picture we have called toxic compares well with that noted by Rolleston [44] and Felty and Keefer.[2] However, there appear to be only scattered references in the literature [10f] to a fall of blood pressure and shock-like appearance. Since 15 of the patients in this series showed this shock-like picture, it cannot be considered a rare occurrence in bacteremia due to Gram-negative bacilli.

The pathophysiologic factors responsible for this shock-like picture remain unknown. However, some evidence can be compiled from this series to show that the picture cannot be considered so-called hepatorenal or even renal in origin. The majority of the patients in this series had normal liver function, and Lichtman,[45] who suggests the term be abolished, states that the hepatorenal syndrome results from abnormal products that are present after severe liver damage. Several of the patients (C8, A6, A2, Pr3, Pr4) with the shock-like picture maintained normal urine output, which is evidence that the picture is not entirely due to renal damage.

A highly speculative explanation of the fact that these patients presented two distinct clinical pictures is that one group of patients may have been reacting primarily to the invading bacteria, while the other group may have suffered predominantly from the effects of an endotoxin. Whatever their explanations, the two pictures did not seem due to any one species of Gram-negative bacilli, since each species caused both clinical pictures.

The apparent malevolence of Proteus bacilli in this series is worthy of comment. The relatively high resistance of this species to most antibiotics makes it entirely possible that infections due to this organism will increase in frequency. It is of interest also that the leucocyte response to Proteus seems lower than that to the other organisms. Does this mean that in this one mode of virulence Proteus has already evolved toward the leucopenic characteristics of Salmonella typhosa?[21]

On the other hand, the relative mildness, in this series, of the bacteremias due to Ps. aeruginosa was in contrast to the cases in the literature.[46]

As was pointed out by Felty and Keefer,[2] the natural course of the infections seemed to be dependent on the underlying disease. However, several cases (C8, S1) were illustrative of how rapidly lethal presumably large doses of Gram-negative bacilli can be.

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44. Rolleston, H. D.: Discussion on the Pathogenic Effect of B. Coli, Brit. M. J. **2**:1186-1192, 1911.

45. Lichtman, S. S., and Sohval, A. R.: Clinical Disorders with Associated Hepatic and Renal Manifestations with Special Reference to So-Called Hepatorenal Syndrome, Am. J. Digest. Dis. **4**:26-31, 1938.

46. Brill and Libman.[4] Eastman and Keene.[5] Stanley.[17]

SUMMARY AND CONCLUSIONS

Twenty-nine cases of bacteremia due to Gram-negative bacilli other than the Salmonella organisms are presented.

The commonest portal of entry was the genitourinary tract, and the commonest precipitating factors were catheterization and instrumentation.

Two distinct clinical pictures were seen. In one the patients appeared toxic and in the other shock-like. The patients suffering from bacteremia due to Proteus appeared to be the most severely ill.

The majority of patients exhibited a marked febrile and leucocytic response.

A therapeutic approach to patients in whom bacteremia due to Gram-negative bacilli is suspected is presented. Emphasis is placed on nonspecific as well as specific therapy.

Examples are given of the use of the laboratory studies in the management of these patients.

The possible roles of antimicrobial therapy in indirectly causing certain types of infection and in bacterial evolution are discussed.

PROTOCOLS OF FIVE OF THE CASES OF BACTEREMIA DUE TO GRAM-NEGATIVE BACILLI

CASE C7.—*Severe bacteremia of E. coli.*

The patient was a 48-year-old white man who was conscious but prostrate when he entered the Minneapolis General Hospital. The history revealed that he had been discharged from this hospital two weeks before, after having recovered uneventfully from a Whipple operation (radical excision of ampulla of Vater) performed for carcinoma of the pancreas. His diabetes had been well controlled with use of 38 units of NPH insulin a day. Thirty-six hours before admission the patient had a chill lasting about one-half hour. He then felt well until six hours before admission, when he had a hard, shaking chill and complained of severe headache. He became unresponsive and was brought to the hospital by ambulance. He was given 20 ml. of 50% dextrose solution on the way to the hospital and was able to answer questions on admission. However, he felt "too weak to move."

Results of Physical Examination.—The temperature was 106 F., the pulse rate, 140, the respiratory rate, 40, and the blood pressure, 50/30. The patient appeared acutely ill but was responsive and able to carry on a calm conversation. Pertinent findings were hot, dry skin, fresh well-healed abdominal scars, and some fulness and tenderness in the midepigastrium.

No urine was obtained for analysis. Studies of the blood showed 13.9 gm. of hemoglobin and 4,000 white blood cells, with 95% polymorphonuclear leucocytes. Blood sugar measured 238 mg. per 100 ml. The carbon dioxide-combining power was 51 vol. %. Blood urea nitrogen was 18 mg. per 100 ml., and chlorides were 107 mEq. per liter. A culture yielded 28 colonies of E. coli per milliliter of blood. A flat plate of the abdomen showed a normal condition. The electrocardiogram was also normal.

Course.—Within the first 12 hr. after admission, the patient was given the following by vein: 1,500 ml. of plasma, 1 liter of whole blood, 1 liter of saline solution, 20 ml. of adrenal cortex extract, and 1,000,000 units of penicillin. In addition, the foot of the bed was placed on shock blocks, and he was given tepid sponges. At the end of this first 12-hour period the temperature fell to 98.6 F. (oral); the pulse rate was 95; the respiratory rate was 25, and the blood pressure, 70/55. Except for the fall in temperature, there was no apparent change in the patient's clinical condition. He remained anuric. Laboratory studies at this time showed 11.2 gm. of hemoglobin and 50,000 white blood cells, with 95% polymorphonuclear leucocytes. Serum bilirubin at 1 minute measured 1.2 mg. per 100 ml., with a total of 3 mg. During the next 12 hours the following were administered by vein: 1.25 gm. of aureomycin, 1 liter of saline solution, 500 ml. of plasma,

and 500 ml. of whole blood. Eighteen hours after admission the patient voided 150 ml. of urine. Culture of this revealed E. coli. The blood pressure was now 74/54. After this, the patient slowly but steadily improved. His blood pressure rose to about 100/70, where it stayed. The urine output increased to a high of 4.8 liters on the sixth hospital day and then remained at about 2 liters per day. The white blood cell count returned to normal, and hemoglobin concentration was maintained at 14 gm. Jaundice disappeared on the fifth hospital day. Aureomycin administration was continued by mouth for 45 days. Repeated blood cultures remained sterile. After an uneventful hospital stay, the patient was discharged, apparently none the worse for his acute illness.

Comment.—The question in this case is, To which of the measures that the patient was subjected to did he respond? It is conceivable that the nonspecific therapy might have produced the favorable response, but it seems more likely that the antibiotics were concerned. Of particular interest is the question of whether the penicillin or aureomycin produced an antitoxin effect and whether, in addition, the aureomycin acted as a diuretic.[47] In this connection it can be noted that diuresis started after the infusion of 1 gm. of aureomycin, in spite of the fact that there was no change in blood pressure.

CASE A2.[48]—*Bacteremia of A. aerogenes treated successfully with Q-19 after therapy with penicillin, aureomycin, chloramphenical, and streptomycin had failed.*

The patient was a 75-yr.-old white man who entered the University of Minnesota Hospital for perineal prostatectomy. The past history revealed that the patient had first noted symptoms of urinary retention two years before admission and had kept an indwelling catheter in place since that time. The catheter had been changed every three weeks. Results of physical examination on admission were essentially normal, except for benign prostatic hypertrophy. The heart rate was regular, and the blood pressure was 150/86. A perineal resection of the prostate gland was performed on the fourth hospital day. The patient had an uneventful postoperative course, except for persistent low-grade fever. On the sixth postoperative day the patient suddenly had a hard, shaking chill and became cyanotic. Physical examination at that time revealed the temperature to be 102 F.; the pulse rate was 120 and irregular; the respiratory rate was 40, and the blood pressure was 50/40. The patient was pale, cyanotic, dyspneic, and comatose. Auricular fibrillation and abdominal distention were present. Laboratory studies showed 11.9 gm. of hemoglobin, 24,000 white blood cells, 92 mEq. of chlorides per liter, and a carbon dioxide-combining power of 22 vol. %. Culture yielded A. aerogenes.

Course.—The patient was receiving penicillin, streptomycin, and sulfadiazine at this time, and their administration was continued. In addition, digitalis, an infusion of 1,000 ml. of 5% dextrose solution in water, a blood transfusion, and oxygen were administered. The foot of the bed was elevated on shock blocks. Some improvement seemed to follow, and the blood pressure rose to 110/70. The next day the patient became jaundiced and remained febrile. A pour plate of 1 ml. of blood grew 23 colonies of A. aerogenes. The penicillin, streptomycin, and sulfadiazine therapy the patient had been receiving was discontinued, and an infusion containing 3 gm. of aureomycin was administered. The following day the patient was still jaundiced and was barely responsive, although his blood pressure remained at 110/60. For the third time A. aerogenes was grown from his blood. His blood urea nitrogen had risen from 16 to 70 mg. per 100 ml. The fourth day after the acute episode the patient remained the same. A fourth blood culture was positive for A aerogenes. Serum bilirubin was 3.6 mg. per 100 ml. in 1 min., and the total was 8 mg. per 100 ml. Plasma hemoglobin was 20 mg. per 100 ml. The results of an in vitro test of the sensitivity of A. aerogenes to antibiotics became available at this time. They showed the strain to be resistant to penicillin, streptomycin, and aureomycin but sensitive to 1.5 γ per milliliter of Q-19. Twenty milligrams of Q-19 was administered intramuscularly every four hours. There was an immediate clinical response. The patient became oriented; his jaundice lessened; the abdominal distention disappeared, and within 24 hours

47. Harned, B. K.; Cunningham, R. W.; Clark, M. O.; Cosgrove, R.; Hine, C. H.; McCauley, W. J.; Stokey, E.; Vessey, R. E.; Yuda, N. M., and Subbarow, Y.: The Pharmacology of Duracillin, Ann. New York Acad. Sc. **51**:182-210, 1948.

48. This patient was treated by Dr. A. Braude, who kindly has permitted this report.

his appearance changed from that of impending death to that of relative well-being. Q-19 treatment was continued for 12 days. During that time four blood cultures were sterile; the blood urea nitrogen dropped from 70 to 24 mg. per 100 ml., and the jaundice disappeared. He continued to have a low-grade fever until the Q-19 therapy was discontinued. During the next month in the hospital, the patient's blood remained sterile and he made steady clinical improvement. He was seen in a follow-up clinic one year after discharge and had remained well and active.

CASE Pr2.—*Bacteremia and probable endocarditis due to Proteus, treated successfully with the combination of chloramphenicol, penicillin, and streptomycin.*

The patient was a 47-year-old white man who was found unconscious at home and brought by ambulance to the Minneapolis General Hospital. The past history revealed that he was a morphine addict of many years' standing and had had "liver trouble" for eight years. At a later date the patient was able to reveal that he had awakened with a severe headache 24 hours before admission and then lost consciousness.

On physical examination the temperature was 104 F.; the pulse rate, 130; the respiratory rate, 38, and the blood pressure, 102/64. The patient was an acutely ill, poorly nourished man in semicoma. Pertinent physical findings were nuchal rigidity, palmar erythema, occasional spider nevi, and petechial hemorrhages over the arms, trunk, and soles. No heart murmur was heard during the initial examination.

Urinalysis revealed the specific gravity to be 1.015, and the pH, 6. There was a 1+ reaction for albumin and a 4+ reaction for red cells. Four to five white blood cells were seen per high-power field. Culture of the urine was sterile. The hemoglobin concentration of the blood was 15.9 gm. White blood cells numbered 8,500, with 83% polymorphonuclear leucocytes. Urea nitrogen was 41 mg., albumin, 2.8 gm., and globulin, 1.9 gm., per 100 ml. Culture of the blood was also sterile. The cerebrospinal fluid was slightly cloudy and contained 87 cells, with 92% polymorphonuclear leucocytes. A smear revealed no pathogens, and culture was sterile.

Course.—A clinical diagnosis of fulminating meningococcic infection was made, and the patient was given 5 gm. of sulfadiazine and 200,000 units of penicillin by the intravenous route. In addition, the foot of the bed was placed on shock blocks, oxygen administration was started, and an infusion of 5% dextrose solution in distilled water was given. Although the patient became conscious, there was no other tangible evidence of clinical improvement. During the first week in the hospital, penicillin and sulfadiazine therapy was continued, but the temperature ranged from 103 to 104 F., the blood pressure remained low, and the patient appeared acutely ill. Because of the lack of clinical improvement, the sulfadiazine treatment was discontinued and aureomycin administration was started. During a seven-day course of aureomycin therapy, the temperature ranged from 100 to 103 F. On the 14th hospital day, a harsh systolic, apical murmur was first heard and new petechiae were noted on the soles. The clinical diagnosis was changed to that of acute bacterial endocarditis. All chemotherapy was discontinued in order to determine the causative organism. The first two blood and urine cultures taken after this were sterile, but the third culture of both the blood and urine grew Proteus. The patient continued to be acutely ill, and the sixth day after specific chemotherapy had been stopped his temperature rose to 106 F. His blood pressure dropped, and his urine output decreased markedly. Penicillin and streptomycin administration was started empirically as the patient was again unresponsive and appeared moribund. Three pints (1,420 ml.) of blood also was given. As soon as the results of the blood and urine cultures were known, chloramphenicol was added to the other two antibiotics. There was a definite clinical response to these measures, and within a 72-hour period the temperature became normal, the blood pressure rose, and the patient responded. However, at this time the blood urea nitrogen was found to be 126 mg. per 100 ml.; so the streptomycin treatment was discontinued. The patient remained improved but became febrile again. In vitro tests of the sensitivity of the strain of Proteus isolated from the blood and urine revealed growth of the organism to the inhibited by the following concentrations of antibiotics per milliliter; aureomycin 100 γ; penicillin 12 units; chloramphenicol 6 γ; combined penicillin and chloramphenicol, 3 units of penicillin and 3 γ of chloramphenicol; combined penicillin, chloramphenicol, and streptomycin, 2 units of penicillin and 2 γ each of the two other drugs. In view of these results streptomycin was again added to the penicillin and chloram-

phenicol combination used in treatment. The patient became afebrile and evidenced further clinical improvement. He was given a 55-day course of combined chemotherapy, which consisted of a daily dose of 2,800,000 units of penicillin, 2 gm. of chloramphenicol, and 1 gm. of streptomycin. On the 37th day of this course of combined therapy the temperature spiked to 99.5 F. (oral). A blood culture drawn at this time grew a hemolytic Enterococci (Group D Streptococcus). In vitro tests of the sensitivity of this organism revealed it to be inhibited by the following concentrations of antibiotics per milliliter: chloramphenicol, 12 γ; aureomycin, 50 γ; penicillin, 6.3 γ; penicillin, streptomycin, and chloramphenicol combined, 2 units of penicillin and 2 γ each of the other two antibiotics. In spite of this incident the patient maintained a steady rate of improvement. His blood urea nitrogen slowly returned to normal; his sedimentation rate went from 103 mm. per 60 min. to 33 mm., and his heart murmur remained constant. At the end of the course of antibiotic therapy he was observed in the hospital for seven weeks. Eight blood cultures drawn during this time were sterile, and the patient was up and about the ward. Six months after discharge the patient was clinically well.

Comment.—Since the patient recovered, there was no way to establish positively the etiological relationships in this case. The two most reasonable possibilities are, first, that this was a case of acute Proteus endocarditis and that the enterococci isolated some weeks later were a contaminant, and, second, that it may have been a mixed endocarditis. At any rate, both organisms isolated from the blood stream were the most sensitive to the combination of the three drugs used. The apparent potentiation between penicillin and chloramphenicol that occurred was investigated further and found to be statistically significant.[40]

CASE Ps4U1.[49]—*Bacteremia of Pseudomonas and an unidentified Gram-negative bacillus, treated successfully with the combination of aureomycin and neomycin.*

The patient was a 3½-year-old white girl who was transferred to Minneapolis General Hospital for preoperative care prior to an ileostomy closure. Past history revealed that she had been operated on because of a ruptured appendix two months before admission. Since that time she had had two additional operations, one because of a partial evisceration and the development of a fecal fistula and the other for the removal of gangrenous ileum and the establishment of a double-barreled ileostomy. The patient had been having recurrent chills and fever during her entire course and had been a difficult problem in nutrition. She had received large doses of penicillin, aureomycin, streptomycin, and chloramphenicol.

On physical examination the temperature was 102 F.; the pulse rate, 140; respiratory rate, 25, and the blood pressure, 102/66. Pertinent findings were evident severe malnutrition, a double-barreled ileostomy opening in the right lower quadrant, a widely opened midline incision which appeared infected and undermined, and mild jaundice.

On laboratory studies the urine was normal. The hemoglobin concentration of the blood was 8 gm. per 100 ml. The white blood cell count was 17,000, with 55% polymorphonuclear leucocytes. The urea nitrogen was 5 mg. per 100 ml.

Course.—By means of cutting down on a vein, fluid electrolytes, and dextrose solution were administered in abundance. The patient was put on a Bradford frame, and special-duty nurses were retained on a round-the-clock basis. Chloramphenicol therapy was started on admission, and the patient's condition was static during the first two days in the hospital. On the third hospital day, she suffered a hard shaking chill and her temperature rose to 104 F. Her blood pressure was unobtainable. She was cyanotic, and her pulse rate was 200 a minute. The leucocyte count was 27,000, with 83% polymorphonuclear cells. A blood culture grew an unclassified Gram-negative coccic bacillus.[26] Aureomycin, streptomycin, cortisone, and oxygen therapy was begun. The following day another severe chill was followed by an elevation in temperature to 105 F. Blood drawn at this time for culture grew 10 colonies of Ps. aeruginosa per milliliter of blood placed on a pour plate. During the next three days the patient did not have as high an elevation of temperature. Her blood pressure stabilized at about 80/50. The aureomycin and cortisone therapy was discontinued at this time, but the administration of chloramphenicol and streptomycin was continued. On the 12th hospital day the patient again

49. Dr. Thersa Haddy and Dr. John Tobin, of the Pediatric Service of the Minneapolis General Hospital, kindly allowed me to follow this patient.

had a hard, shaking chill, and her temperature rose to 104 F. The unclassified bacillus was grown from her blood at this time. For the next 27 days the patient had at least one and usually two or three shaking chills a day, with her temperature usually rising to 104 to 106 F. Four of five blood cultures made during this time grew the unclassified bacillus. The leucocyte count ranged from 15,000 to 57,000. The hemoglobin concentration dropped approximately 1 gm. per day, and much difficulty was experienced in giving blood transfusions. Even carefully washed cells seemed to cause an immediate shock-like reaction. At one point the hemoglobin level dropped to 6 gm. per 100 ml. Finally, it was found that 20 mg. of cortisone given just before the transfusion seemed to allow the blood to run in without incident, and transfusion became possible. In vitro sensitivity tests of the strain of the unclassified bacillus that was constantly isolated from the blood stream revealed the following results:

Antibiotics	Inhibitory Concentration, γ/ml.
Chloramphenicol	100
Aureomycin	12.1
Penicillin	100 (units/ml.)
Neomycin	1.5
Streptomycin	25
Polymyxin B	1.5
Chloramphenicol and polymyxin	1.5 of each
Neomycin and polymyxin	0.38 of each
Neomycin and aureomycin	0.38 of each

The patient was given the following chemotherapy: chloramphenicol, 2 gm. a day for 11 days; streptomycin, 0.75 gm. a day for 11 days; aureomycin, 200 mg. a day intravenously for 7 days; neomycin, 0.75 gm. a day for 24 days; polymyxin B, 30 mg. a day for 19 days. Usually more than one drug was given at a time. Administration of the combination of polymyxin and neomycin was continued for 19 days. The only evidence of a possible response to this medication was that the Ps. aeruginosa disappeared from both the blood and the urine. Finally, when an in vitro sensitivity test revealed a possible synergism between aureomycin and neomycin, administration of these drugs was started in combination. Slow but steady improvement seemed to follow the institution of this regimen. The severe chills and fever abated, and the temperature gradually came down. After a 17-day course of this combined therapy the hemoglobin concentration had been built up to 15 gm., and the leucocyte count ranged from 13,000 to 18,000, with about 75% polymorphonuclear cells. Three blood cultures had been sterile. At this point it was felt that the surgical measures should be risked. With the patient under ether anesthesia a two-layer closure of the ileostomy opening and a closure of the other abdominal incision were performed. The immediate postoperative course was uneventful, and the child started to pass feces by rectum within 48 hr. after the operation. On the second postoperative day it was noted that the child had become deaf; therefore, the neomycin and aureomycin therapy was discontinued. Chloramphenicol administration was continued by mouth. Three weeks postoperatively the patient was afebrile, passing feces by rectum, and eating and drinking well. The leucocyte count was 10,000, with 78% polymorphonuclear cells, and the blood pressure had risen to 110/80. When seen six weeks later, the child was up and about the ward with no complaints. With the exception of continued deafness, the patient was well four months after discharge from the hospital. Hearing had not returned.

CASE S1.—*A fulminating bacteremia due to Eberthella oedematiens.*

The patient was a 74-year-old white man who was brought to the Minneapolis General Hospital in coma. History revealed that he had lived alone and had been found unconscious on the floor by a neighbor. He had apparently been feeling fairly well when he went to bed the evening before admission.

Physical examination revealed the temperature to be 96 F. The pulse rate was unobtainable. Respirations numbered 24. The heart rate was 136 and irregular. The blood pressure was 60/40. The patient was an obese man who appeared moribund. Pertinent physical findings were spider angiomas over the forehead and thorax, petechiae and ecchymoses over the lower part of the legs and the hands, and auricular fibrillation. The liver was palpable and hard.

Laboratory studies of the urine disclosed a specific gravity of 1.020, a pH of 6, a 1+ reaction for albumin, and six to eight granular casts and an occasional white blood cell per high-power field. Studies of the blood showed the hemoglobin concentration to be 12 gm. per 100 ml. and the white-blood-cell count, 2,700 per cubic millimeter, with 82% polymorphonuclear cells. The blood sugar was 23 mg. per 100 ml. The carbon dioxide-combining power was 38 vol. %. Urea nitrogen measured 50 mg. per 100 ml., and chlorides were 111 mEq. per liter. The cephalin-cholesterol flocculation was 4+. Bilirubin at 1 min. was 2 mg. per 100 ml., with a total of 4.5 mg. There were 1.8 gm. of albumin and 3 gm. of globulin. A culture yielded Eberthella oedematiens.[25] The spinal fluid contained 1,533 cells, 85% of which were polymorphonuclear forms, and 24 mg. of sugar per 100 ml. A smear of the fluid was loaded with Gram-negative rods. Culture yielded Eberthella oedematiens.

Course.—The patient was given infusions containing 1 gm. of aureomycin, 3 liters of blood, 1 liter of 10% dextrose solution, and 0.6 mg. of digitoxin. In spite of this therapy he died five hours after admission. Postmortem examination revealed ruptured duodenal ulcer, purulent meningitis, cirrhosis of the liver, generalized peritonitis, and splenomegaly. Eberthella oedematiens was cultured from peritoneal exudate obtained at postmortem examination.

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TREATMENT OF PNEUMOCOCCIC MENINGITIS WITH PENICILLIN COMPARED WITH PENICILLIN PLUS AUREOMYCIN

Studies Including Observations on an Apparent Antagonism Between Penicillin and Aureomycin

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PNEUMOCOCCIC meningitis is well known as one of the most serious forms of bacterial meningitis. Before the sulfonamides were used pneumococcic meningitis was almost always fatal. With the advent of penicillin an era of more successful therapy was started. Originally it was established that when small systemic doses of penicillin were given the result could be greatly improved by administering the antibiotic intrathecally. It was later shown [1] that the administration of large doses of penicillin systemically gave still better results and that intrathecal penicillin therapy was unnecessary when this regimen was employed. In spite of these advances, however, approximately one of three persons with pneumococcic meningitis still died, as shown on analysis of series of unselected cases. Consequently, when aureomycin became available and was shown to be effective in therapy of isolated cases of pneumococcic meningitis, we decided to test its efficacy by treating alternate patients with either penicillin alone or a combination of penicillin and aureomycin.

After this study had been in operation for some time, Jawetz published several reports which demonstrated that chloramphenicol exerted an antagonistic action on the effect of penicillin against Streptococcus faecalis,[2] beta hemolytic Streptococcus, and Klebsiella [3] in vitro and in mice. When we reviewed the results of our alternate-case-treatment study, we found that the case-fatality rate among the patients who received penicillin plus aureomycin had not fallen below the fatality rate for the patients who received penicillin alone but was actually higher. The present paper presents an analysis of the two groups of patients, together with a discussion of the significance of our findings.

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3. Jawetz, E.; Gunnison, J. B.; Speck, R. S., and Coleman, V. R.: Studies on Antibiotic Synergism and Antagonism, A. M. A. Arch. Int. Med. **87**:349-359 (March) 1951.

METHOD OF STUDY

Included in the present analysis are all patients with pneumococcic meningitis who have been treated by one or both of us during the last five years with doses of 1,000,000 units of crystalline penicillin given intramuscularly every two hours. Forty-three of these received penicillin alone, 21 of whom were reported on previously by Dowling and his associates.[1] Fourteen patients received the combination of aureomycin hydrochloride and penicillin. Aureomycin was given intravenously at six-hour intervals for the first two to four days of therapy. The adult patients were given 0.5 gm. per dose and the children 12.5 mg. per kilogram of body weight. Several of the patients received aureomycin by mouth as well as intravenously, and, during the recovery phase of the illness, the oral route was employed exclusively. Oral doses were 0.5 gm. every three hours for adults and 50 to 100 mg. per kilogram of body weight per 24 hr. in divided doses for children.

The patients treated with the combination of aureomycin and penicillin were alternated with the last 14 patients treated with penicillin alone. (Hereafter these will be designated as the "penicillin-aureomycin group" and the "penicillin control group," respectively, while the entire group of 43 patients who were treated with penicillin alone will be designated as the "penicillin group.")

Since the results obtained in this smaller control group were similar to those obtained in the entire group of patients treated with penicillin alone, they have not been analyzed separately except with respect to the case-fatality rate.

RESULTS

In Table 1 the case-fatality rates of the patients in the penicillin-aureomycin group are compared with the fatality rates of the patients treated with penicillin

TABLE 1.—*Case-Fatality Rates in Pneumococcic Meningitis in Relation to the Ages of the Patients*

	Patients Treated with Penicillin			Patients Treated with Penicillin and Aureomycin		
		Died			Died	
Age, Yr.	No.	No.	%	No.	No.	%
Less than 1	5	3	60	0	..	..
1 to 13	6	0	0	2	0	0
14 to 49	15	3	20	6	5	83
50 and over	17	7	41	6	6	100
All ages	43	13	30	14	11	79

alone, arranged according to ages of the patients. When patients of all ages are considered, the fatality rate was much lower among the patients who received penicillin alone (30%) than among those who received both antibiotics (79%). This difference is highly significant when tested by the Yates modification for small series of the chi-square test ($\chi^2 = 8.3$). Moreover, if only the controlled portion of the series is considered, three among 14 penicillin-treated patients died (21%). Again this difference, tested by the same method, is found to be significant ($\chi^2 = 7.0$).

In the separate age groups the fatality rate was consistently higher among the patients treated with penicillin and aureomycin than among those who were given penicillin alone. The differences in the fatality rates in the group 14 to 49 years of age and in the age group over 49 years were each of statistical significance. (The chi-square values in these groups, calculated by the Yates modification, were 4.8 and 4.9, respectively.) No deaths occurred in either series in the most favorable age period, 1 to 13 years. If the age groups with less favorable prognoses are combined

so that all patients under 1 year and over 49 years of age are included together, 22, or 51%, of the penicillin-treated patients and six, or 43%, of the penicillin-aureomycin-treated patients, fell into these groups. Any weighting which occurred on the basis of age, therefore, tended to place more penicillin-treated patients than penicillin-aureomycin-treated patients in these unfavorable age groups.

In Table 2 is shown the effect of certain prognostic factors upon the case-fatality rates in the patients treated by the two methods. We have found the number of organisms seen in the initial smear of the centrifuged sediment of the cerebrospinal fluid to be an important factor in prognosis. Although this information was not recorded in every case, it is apparent from Table 2 that a larger proportion of the penicillin group than of the penicillin-aureomycin group fell into the category with the poorest prognosis (four or more pneumococci per oil-immersion field). Again, the fatality rates were higher in each category for the patients treated with the combination of aureomycin and penicillin than for the patients treated with penicillin alone.

TABLE 2.—*Case-Fatality Rates in Pneumococcic Meningitis in Relation to Certain Prognostic Factors*

Prognostic Factors		Patients Treated with Penicillin				Patients Treated with Penicillin and Aureomycin			
				Died				Died	
		No.	% of Total	No.	%	No.	% of Total	No.	%
Number of pneumococci (per oil-immersion field)	Many (4 or more)	14	33	7	50	3	21	2	67
	Few (1 to 3)	3	7	3	0	7	50	5	71
	None (identified by culture only)	1	2	0	0	2	14	2	100
	Not recorded	25	58	6	24	2	14	2	100
Status of sensorium on admission	Comatose	15	35	8	53	2	14	2	100
	Not comatose	28	65	5	18	12	86	9	75
Infectious foci elsewhere than in meninges	Present	32	75	13	41	9	64	9	100
	Absent	11	25	0	0	5	36	2	40
Other diseases	Present	11	26	6	55	4	29	3	75
	Absent	32	74	7	22	10	71	8	80
Day of disease on which treatment was started	1st or 2d	14	33	3	21	6	43	5	83
	3d or 4th	11	25	3	27	7	50	5	71
	5th or later	8	19	4	50	0	..	..	..
	Unknown	10	23	3	30	1	7	1	100

The grave prognostic significance of the presence of coma is confirmed by the next set of figures in Table 2. In the group of patients treated with penicillin, death occurred in 53% of the patients who were admitted to the hospital in coma, whereas death occurred in only 18% of the patients who were not in coma on admission. The corresponding figures for patients in the penicillin-aureomycin group were 100 and 75%, respectively. Again, the penicillin group contained a higher percentage of patients in the category which offered the poorest prognosis, since 35% of the patients in this group were comatose on admission, as compared with only 14% of the patients in the penicillin-aureomycin group.

Pneumococcic infections occurred in localities other than the meninges in 75% of the patients in the penicillin group and in 64% of the patients in the penicillin-aureomycin group. The fatality rates were 41 and 100%, respectively, for the two groups. Although the fatality rates were lower when no focus was present elsewhere, they were still higher for the penicillin-aureomycin group than for the penicillin group (fatality rates: 40 and 0%, respectively).

Other diseases were present in approximately the same percentage of the patients in the two therapeutic groups, but again the fatality rate was worse in the combined treatment group (75%) than in the group of patients treated with penicillin alone (55%). The complicating diseases included arteriosclerotic or hypertensive heart disease, alcoholism, cirrhosis of the liver, diabetes mellitus, syphilis, and nephrosis.

Delay in the initiation of treatment is an important factor in increasing the death rate in pneumococcic meningitis as in most infectious diseases. Among the patients treated with penicillin the fatality rate rose progressively from 21%, for those whose treatment was started during the first two days of the disease, to 50%, for those who did not receive penicillin until the fourth day or later. On the other hand, the fatality rates among the patients receiving aureomycin and penicillin were higher, regardless of the time that therapy was started.

Other data may be helpful in evaluating the effectiveness of the two therapeutic regimens. Among the 13 patients who died during treatment with penicillin alone, nine, or 69%, died within 48 hours after the initiation of treatment, whereas only three of the 11 patients who died while receiving the combination of antibiotics died during the first 48 hours. On the other hand, when the number of deaths is considered in relation to the total number of patients treated, an identical proportion of patients died within 48 hours in each treatment group, namely, 21%. This would seem to indicate that penicillin usually failed to bring about recovery only in acute fulminating cases, whereas the penicillin-aureomycin combination, while it failed to have a beneficial effect in the same proportion of patients with an acute fulminating course, was also unsuccessful in a number of patients with less acute and presumably less severe infections.

Among the patients who recovered, the average duration of fever in the penicillin-treated group was 4.6 days, with a standard deviation of 3.6, and in the combination group the corresponding figures were 13 days and 8.5, respectively. This difference is significant by the t method ($t = 3$).

The duration of pleocytosis of the cerebrospinal fluid after the start of therapy was 13 and 20 days for the penicillin and the penicillin-aureomycin groups, respectively. This difference was not statistically significant because of wide variations among the different patients.

COMMENT

When new methods of treatment become available, it is necessary that they be incorporated as rapidly as possible among existing therapeutic procedures. Since pneumococcic meningitis was a condition in which the presently available methods of treatment were not completely satisfactory, it offered an excellent situation in which to evaluate the efficacy of aureomycin as an adjunct to penicillin. From the results obtained, it seems clear that the addition of aureomycin detracted from the therapeutic efficacy of penicillin in this disease. The death rate of patients treated with aureomycin in addition to large doses of penicillin was more than twice as high as that of patients treated with the same doses of penicillin alone, whether a strictly alternated control group of patients was used for comparison or a much larger group of patients who were found to be comparable in all respects. Furthermore, recovery occurred more rapidly in the patients treated with penicillin alone, as judged by the rapidity of the decline in fever and in the leucocyte count of the cerebrospinal fluid. Several possible explanations for this phenomenon may be adduced:

First, the groups may not be comparable. However, when the two groups were analyzed with respect to the factors known to influence prognosis, such as age, number of pneumococci in the spinal fluid, the presence or absence of coma, of other purulent complications, or of underlying diseases, and the duration of disease before therapy, it was found that there was little difference between the two groups. The differences which were present made for a poorer prognosis in the penicillin group, in that a larger percentage of the penicillin-treated patients were comatose on admission or were treated late. Without exception, the results of treatment with penicillin were superior to those with the combination in every situation in which comparison was made.

In addition, when death occurred in patients receiving penicillin and aureomycin, it usually took place several days after the initiation of therapy. Since there were equal percentages of patients dying within the first 48 hours of the start of therapy in the two groups, these delayed deaths almost certainly represent treatment failures. For the reasons cited above, we do not believe that inequalities in the groups account for the different results obtained with the two therapeutic regimens.

A second possibility is that aureomycin in the doses used may be toxic, particularly to the liver,[4] and so contribute to the deaths. In the case of one patient, in whom jaundice developed during the course of penicillin-aureomycin therapy, we believe that such a toxic reaction may possibly have contributed to the fatal outcome. In no other patient, however, was there evidence of hepatic damage. Moreover, we have seen no evidence of toxic effects in the 43 patients with other etiologic types of meningitis whom we have treated with similar doses of aureomycin, and only two of these patients died. For these reasons we do not feel that the unfavorable results in the penicillin-aureomycin group were caused by toxic effects of aureomycin.

A third possible explanation is that aureomycin and penicillin may have an antagonistic effect upon each other when used in combinations. Such a phenomenon has been shown to occur in vitro and in vivo in certain mouse infections by Jawetz [5] when chloramphenicol was used, and by us [6] when aureomycin was employed. It is interesting to note that we have treated three patients having pneumococcic meningitis with aureomycin alone with good results. One of these patients did relapse, however. Others have reported the successful treatment of pneumococcic meningitis with aureomycin alone.[7] Such results suggest, at least, that, in addition to the evident interference with penicillin activity by aureomycin, penicillin interferes with the antibacterial effects of aureomycin. This seems to us to be the most likely explanation for the phenomenon which we have observed.

The present study further confirms the efficacy of the use of large systemic doses of penicillin in the treatment of pneumococcic meningitis without the necessity of

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5. Gunnison and others.[2] Jawetz and others.[3]

6. Dowling, H. F.; Lepper, M. H., and Roth, E.: Unpublished data.

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intrathecal therapy.[1] Over a period of five years this regimen has produced consistently good results in our hands. The major problems now are the patients who are comatose, and especially those who are moribund, on admission. Earlier diagnosis would seem to be the only effective answer to this problem at present.

SUMMARY

Among 43 patients with pneumococcic meningitis treated with massive parenteral doses of penicillin, 13 (30%) died. Among 14 similar patients treated with aureomycin in addition to the same dose of penicillin, 11, or 79%, died. The last 14 of the 43 penicillin-treated patients were treated alternately with the 14 penicillin-aureomycin-treated patients. Among this control group of penicillin-treated patients there were three (21%) deaths.

There was no essential difference between the two groups with respect to age, the number of organisms in the spinal fluid, mental status, portal of entry, pneumococcic complications, the presence of other diseases, or the day on which therapy was started. It is concluded that the poor results with the penicillin-aureomycin combination were not due to the chance inclusion of more severely ill patients in this group, nor did toxicity to aureomycin seem to account for the results. Rather, the evidence seems to favor the conclusion that penicillin and aureomycin are mutually antagonistic when employed together in the treatment of pneumococcic meningitis. It appears unwise to use combinations of penicillin with aureomycin, terramycin, or chloramphenicol in treatment of patients with pneumococcic meningitis, or in treatment of any patients, even those with milder infections in which antagonism has been shown in vitro.

Consistently good results have been obtained in the treatment of pneumococcic meningitis with doses of 1,000,000 units of penicillin at two-hour intervals without use of the intrathecal route. Further investigation of regimens employing insoluble penicillin salts in order to produce similar serum and spinal-fluid concentrations is indicated, since these may simplify the dosage schedule.

Since good results have been obtained by the use of aureomycin therapy alone in pneumococcic meningitis, further studies should be made on the efficacy of aureomycin, terramycin, and chloramphenicol, without adjuvant penicillin, in the treatment of this disease.

Dr. Norman Blatt, Dr. Eston R. Caldwell Jr., Dr. Harold L. Hirsh, Dr. Harold W. Spies, Dr. Paul F. Wehrle, and the late Dr. Lewis K. Sweet assisted in the study of these cases.

PRIMARY HERPES SIMPLEX VIRUS INFECTION OF THE ADULT

With a Note on the Relation of Herpes Simplex Virus to Recurrent Aphthous Stomatitis

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INFECTION of man with the virus of herpes simplex commonly occurs in infancy, when it is usually manifested as an acute stomatitis.[1] According to the widely accepted concept of Burnet and Williams,[2] the primary infantile infection is followed by the latent persistence of virus in the tissues. At times the latent virus may be activated and induce mild recurrent disease in the form of vesicular lesions ("fever blisters") of the lips or other sites invaded during the primary infection. Persons subject to recurrent herpes are found to have measurable concentrations of specific neutralizing antibody in the serum.[3] This seeming paradox has been attributed to the continuing antigenic stimulus arising from the presence of the latent virus. In the majority of adults (65 to 90%), serological evidence indicates previous infection with herpes simplex virus.[4]

Primary herpetic infection of the adult has been only rarely described. Essential to this diagnosis is the demonstration of the presence of specific neutralizing or complement-fixing antibodies during convalescence but not in the acute phase of the illness. The recovery of herpes virus and the demonstration of pathological lesions stressed by earlier authors are of secondary importance in establishing the etiological role of a virus known to be present in most adults. It is the purpose of the present report to describe instances of primary herpetic infection in adults which were associated with disease dissimilar to that identified with the childhood infection and to summarize existing knowledge of primary infection of the adult. In addition, evidence will be presented that herpes simplex virus may be etiologically related to recurrent aphthous stomatitis.

METHODS

Virus recovery and neutralization tests were carried out by the intraperitoneal inoculation of newborn mice. The use of this unusual experimental host was a consequence of investigation of Case 1 for possible infection with Coxsackie virus, an agent usually propagated in infant mice or hamsters. This approach led to the recovery of a strain of herpes simplex virus in infant

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3. Andrewes, C. H., and Carmichael, E. A.: A Note on the Presence of Antibodies to Herpes Virus in Post-Encephalitic and Other Human Sera, Lancet **1**:857, 1930.

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mice and to the subsequent use of such animals in studies with the agent. Detailed investigation of herpes simplex virus infection of infant mice has demonstrated that titrations of specific neutralizing antibody may be performed with ease and good reproducibility when mixtures containing a constant amount of virus and decreasing amounts of serum are inoculated intraperitoneally in 1 to 2-day-old mice.[5] This procedure provides the advantage of a decisive lethal end-point and permits quantitative studies of serum antibody concentration. All neutralizing antibody titers reported in the present paper were determined by this method.

REPORT OF CASES

CASE 1.—*History.*—A 42-year-old white man was admitted to the hospital Dec. 13, 1949, with complaints of marked malaise, anorexia, and cough. Fourteen days before admission, the patient had noted slight malaise and moderate enlargement and tenderness of a left submandibular lymph node. That evening he experienced increased malaise and moderate generalized aching. During the ensuing week the patient noted increasing fatigability, anorexia, and malaise and felt feverish late in the day. Symptoms at this time suggested infectious mononucleosis. About seven days before admission anorexia became severe and was associated with burning epigastric pain, accentuated by ingestion of coffee or acid foods; the diet became altered so that it consisted entirely of toast, milk, and eggs. (While the patient received this diet, light-colored stools were passed frequently.) During the week prior to hospitalization, a low-grade afternoon fever (elevation of temperature to 100 to 100.6 F. [oral]) was recorded. Hyperesthesia and hyperalgesia of the left side of the face were noted during shaving four days before admission. One day prior to admission, a small, painful, punched-out ulcer appeared on the inner surface of the lower lip.

Results of Physical Examination.—The patient was a well-developed, well-nourished white man who did not appear acutely ill. The rectal temperature was 100 F.; the pulse rate, 70, and the respiratory rate, 20. No evidences of upper-respiratory-tract disease were discernible on inspection of the nose, mouth, and throat. Relevant positive findings were as follows: The submaxillary lymph nodes were moderately enlarged but not tender. There were strings of small, nontender, anterior cervical lymph nodes bilaterally, larger on the left. The axillary lymph nodes were moderately enlarged bilaterally, but not tender. The inguinal nodes were also palpable, slightly enlarged, but not tender. The rounded, nontender edge of the liver was just palpable below the right costal margin. The spleen was not palpable. Neurological examination revealed slight hyperalgesia of the skin covering the left deltoid muscle, but no other evidence of nervous system disease.

The diagnoses considered on admission were (1) acute mild hepatitis, (2) infectious mononucleosis, and (3) Coxsackie virus infection.

Laboratory Findings.—White blood cells numbered 9,400, with a differential count of 57% segmented neutrophils, 27% lymphocytes, 13% atypical lymphocytes, 1% monocytes, and 2% eosinophils. The erythrocyte-sedimentation rate (Wintrobe) was 14 mm. in one hour. The Mazzini test gave a negative reaction; the heterophile agglutination was normal. Urinalysis and liver-function tests, including determinations of total protein, zinc turbidity, phenol turbidity, total bilirubin, and sulfobromophthalein (bromsulphalein®) sodium retention, gave normal results. Nose and throat cultures yielded no pathogenic bacteria; a blood culture revealed no bacterial growth. A chest roentgenogram appeared essentially normal.

Course.—Soon after admission the patient's symptoms began to lessen, and he became afebrile on the second hospital day. Hyperesthesia over the left side of the face and deltoid region was remarked as late as the second hospital day, but the patient's progressive improvement permitted discharge for convalescence at home after five days of hospitalization.

Investigation.—Intraperitoneal inoculation of infant mice with a throat washing obtained on the sixth day of illness (and prior to the appearance of the labial ulcer) resulted in the recovery of a filter-passing agent which produced ascending paralysis and death in these animals. This agent was noted to have the host range and serological characteristics of herpes simplex virus. Numerous titrations established that serum samples obtained from the patient before the illness and during its early phase failed to neutralize the homologous strain of virus. In contrast,

5. Kilbourne, E. D., and Horsfall, F. L., Jr.: Studies of Herpes Simplex Virus in Newborn Mice, J. Immunol., to be published.

convalescent and later serum specimens (Table 1) caused neutralization of the homologous virus, as well as other strains of herpes virus, in infant mice and *in ovo*. Serological investigation during the patient's illness revealed no evidence of infection by the viruses of mumps, influenza A, or influenza B, by Taylor's 1233 virus,[6] or the Conn No. 5 strain of Coxsackie virus.

Comment.—It is apparent that this patient suffered a primary infection with herpes simplex virus coincident with a subacute febrile illness resembling infectious mononucleosis. Because this type of syndrome has not previously been related to herpetic infection, it may seem hazardous to suggest an etiological role for the virus of herpes simplex, even with the evident concurrence of the presence of the virus, a specific antibody response, and clinical illness. However, it should be pointed out that certain features of the illness, i. e., facial hyperalgesia and labial ulceration, are compatible with currently recognized clinical patterns of herpetic infection. Furthermore, there is evidence against a coexisting infection with a number of other viruses. Investigation of similar illnesses with attention to their possible herpetic origin is indicated to resolve this problem.

CASE 2.—*History.*—A 21-year-old white man was admitted to the hospital on April 1, 1950, with the chief complaint of severe sore throat. Ten days before admission the patient had noted the appearance of a painful gingival ulcer near the second molar of the left mandible, which persisted for about three days. Three days before admission he noted the gradual onset of sore

TABLE 1.—*Herpes-Simplex-Virus Infections in Adults (Present Cases)*

		Herpes Virus Recovered		Neutralizing Antibody	
Case	Disease	Date of Virus Recovery	Host	Serum Date	Titer*
1	Acute febrile illness	12/ 5/49	Infant mouse	12/ 1/49	0†
				12/29/49	40
				1/ 8/51	15
2	Acute pharyngitis (temperature = 104.6 F.)	4/ 1/50	Infant mouse; chick embryo	4/ 1/50	0†
				4/10/50	43
				4/20/50	91
3	Recurrent aphthous ulcers (see Table 2 for details)	1/ 7/50	Infant mouse; chick embryo	12/13/49	192
				1/24/50	192
		3/ 6/50	Chick embryo	3/ 6/50	128
				3/22/50	256
		5/ 7/50	Infant mouse; chick embryo	12/ 6/50	120

* Antibody titers determined in infant mice (reciprocal of serum dilution); 20 to 50 median lethal doses of herpes virus were employed.
† < 10.

throat, slight malaise, and feverishness. The next morning the patient complained of increased fever and pharyngeal pain. At noon his oral temperature was found to be 102 F., and a physician was called. At approximately 1 p. m., 300,000 units of crystalline penicillin G procaine was administered intramuscularly, followed by a similar injection at 7 p. m., at which time the patient's oral temperature was 104 F. The morning of the day before admission, 600,000 units of the same penicillin preparation was injected when the patient's temperature was again found to be 104 F. During this time the patient's chief complaint continued to be "sore throat," although frontal headache and dizziness were concomitant symptoms. The morning of admission, the patient's temperature was again 104 F. and his physician administered one oral dose of aureomycin (amount unknown). Because of continued high fever and sore throat in the face of massive penicillin therapy, the patient was referred to this hospital for study.

Results of Physical Examination.—Physical examination disclosed a tall, thin, white man, who appeared drowsy and acutely ill, but who was able to reply rationally to interrogation. The rectal temperature was 104.6 F., the pulse rate, 100, and the respiratory rate, 20. Relevant positive findings were as follows: The tongue was dry and coated; the papillae at the base of the tongue appeared hypertrophic. There were no lesions of the buccal mucosa except for a small, superficial ulceration of the lower lip at its dental margin. There was diffuse redness

6. Taylor, R. M.: Studies on Survival of Influenza Virus Between Epidemics and Antigenic Variants of the Virus, Am. J. Pub. Health **39**:171, 1949.

of the posterior pharynx. In the left tonsillar fossa, a shred of tonsillar tissue remained which was the site of several lesions. Similar lesions were also present in the posterior pharynx. These lesions consisted of small red papules with yellow pustular centers. Abutting them were islands of follicular lymphoid hyperplasia. There was minimal but definite nuchal rigidity evident on anterior flexion of the neck. Marked enlargement and tenderness of the left tonsillar node at the jaw angle were present. There were large tender cervical nodes along the posterior border of the left sternocleidomastoid muscle. Smaller and only slightly tender nodes were felt in the corresponding area on the right. The diagnostic impression on admission was infectious mononucleosis or a related illness.

Laboratory Findings.—The white blood cell count was 6,650, with a differential count of 6% band neutrophils, 28% segmented neutrophils, 56% lymphocytes, 6% atypical lymphocytes, and 4% monocytes. The Mazzini reaction was negative. Urinalysis gave normal results. A roentgenogram of the chest revealed no abnormalities. Cultures of material from the nose and throat yielded no pathogenic bacteria, and a blood culture showed no growth.

Course.—In view of the low total leucocyte count, the absence of polymorphonuclear leucocytosis, and the cultural results, it was believed unlikely that the patient's illness was of bacterial origin. Accordingly, he was treated symptomatically with codeine and hot saline irrigations, and no further antimicrobial therapy was administered. The day after admission the patient's temperature fell to 100.5 F. (rectal) and coincidentally his nuchal spasm and drowsiness disappeared. However, palpable cervical nodes, which had not been observed on admission, appeared in the right lower lateral cervical region. By the fourth day of hospitalization the patient had become afebrile, and the following day he was essentially asymptomatic except for generalized weakness; physical examination revealed regression of pharyngeal lesions and lymphadenitis. Seven days after admission he was discharged.

Investigation.—Inoculation of infant mice with a throat washing from this patient resulted in the recovery of a filter-passing agent serologically identified as herpes simplex virus. The virus was subsequently recovered again from the same throat washing in both infant mice and chick embryos. Neutralization tests, using the homologous virus recovered from this patient, demonstrated a progressive rise in the antibody titer of successive serum samples (Table 1). No antibody was detected in serum obtained during the acute phase of illness. Heterophile and cold agglutination, influenza A and B hemagglutination-inhibition, and antistreptolysin-O titrations gave negative results.

Comment.—It appears clear that this patient incurred infection with herpes simplex virus coincident with a febrile illness and that the serological and cultural evidence is against concomitant infection with commonly encountered causes of acute pharyngitis. The ineffectiveness of penicillin therapy and the lack of polymorphonuclear leucocytosis are compatible with a viral infection and lend support to the possibility that the patient's illness was etiologically related to herpes simplex virus infection. Requestioning of the patient elicited denials of a past history of "fever blisters" or "cold sores" but revealed that he was subject to painful small white ulcers of the gums and buccal mucosa. This case appears to represent a primary herpetic pharyngitis of the type commonly seen in infancy, but without the concomitant ulcerative stomatitis usual in such cases.

CASE 3.—A 29-year-old white man had been subject to recurrent lip vesicles and aphthous ulcers of the mouth and gums since childhood. Current controversy [7] concerning the causation of buccal aphthous ulcers instigated investigation of the problem, with the use of the newborn mouse as an experimental host. Injection of throat and mouth washings obtained from this patient prior to January, 1950, had produced no evident disease in infant mice (Table 2).

During the period between January, 1950, and November, 1950, the patient suffered five separate episodes of ulceration of the gums or buccal mucosa. The relevant facts concerning these illnesses are summarized in Table 2. The first episode in January, 1950, was attended by sore throat and malaise. Swabbings from a "punched-out" gingival ulcer produced fatal paralytic disease in newborn mice and typical herpetic lesions on chorioallantoic membranes of chick embryos. Passage materials obtained from both species were neutralized by herpes simplex virus

7. Dodd, K., and Ruchman, I.: Herpes Simplex Virus Not the Etiologic Agent of Recurrent Stomatitis, Pediatrics **5**:883, 1950. Blank, H.; Burgoon, C. F.; Coriell, L. L., and Scott, T. F. M.: Recurrent Aphthous Ulcers, Correspondence, J. A. M. A. **142**:125 (Jan. 14) 1950.

antiserum. Three weeks later, mouth washings were obtained from the patient when he noted small tender sublingual vesicles. No virus was obtained from these washings. In February, 1950, infant mice were inoculated with throat washings taken when the patient was ill with an infection of the upper respiratory tract. No virus was recovered. In March, 1950, after a submucous resection of the nasal septum, the patient experienced soreness of the throat and palate, followed in 48 hours by the appearance of discrete yellow papules on the gums and palate (Fig.). Coincidentally, severe esophageal and gastric burning occurred, resulting in dysphagia which necessitated a bland fluid diet. Material from papules scraped from the palatal mucosa produced pocks on the chorioallantoic membrane but failed to kill infant mice. The agent was filtrable, was passed in series, and was neutralized by herpes simplex virus antiserum. It is noteworthy that for one week prior to operation the patient had noted a painful gingival ulcer adjacent to the palate. In May, 1950, the patient again experienced a gingival ulcer, coincident with symptoms of headache, gingival soreness, nasal burning and otalgia, and submandibular lymphadenitis on the side involved by the ulcer. A virus serologically identified as herpes simplex was recovered both in infant mice and in chick embryos from saliva and swabbings of the ulcer. In October, 1950, attempts to recover virus from two separately occurring ulcers were fruitless.

TABLE 2.—*Relation of Herpes Simplex Virus to Various Illnesses in One Herpetic Subject (Case 3)*

Illness No.	Date of Illness	Clinical Disease	Recovery Virus	Host
1	8/15/49	Common cold (rhinitis)	0	Infant mouse
2	9/29/49	Common cold (rhinitis)	0	Infant mouse
3	10/31/49	Common cold (rhinitis)	0	Infant mouse
4	1/ 7/50	Aphthous ulcer (gingival), sore throat, malaise	+ +	Infant mouse Chick embryo
5	1/24/50	Sublingual vesicles; no ulcers	0	Infant mouse
6	2/14/50	Sore throat, cough, malaise; no ulcers	0	Infant mouse
7	3/ 6/50	Sore throat and palate, yellow papules on palate and gums; antecedent aphthous ulcers	+ 0	Chick embryo Infant mouse
8	5/ 7/50	Headache, sore gingivae, dry rhinitis, submandibular lymphadenitis, otalgia, gingival aphthous ulcers	+ +	Infant mouse Chick embryo
9	10/11/50	Sore throat, otalgia, aphthous ulcer (gingival)	0	Infant mouse
10	10/24/50	Buccal aphthous ulcer	0	Infant mouse

Studies of the subject's herpes-neutralizing antibody titer throughout this period revealed the high antibody concentration characteristic of most adults, but no significant change in titer which could be correlated with the illnesses (Table 1).

Comment.—The recovery of a virus from a patient does not constitute proof of the etiological role of that virus in an illness. Of equal, if not greater, importance is the demonstration of an increase in antibody concentration against the virus during convalescence. It is obvious, however, that such antibody studies in patients subject to recurrent herpes simplex are unlikely to be of value, as it is characteristic of this disease that antibody levels are high at the onset of recurrence. It is, therefore, necessary to rely on the less satisfactory evidence provided by the correlation of lesions with recoverable virus. In the present instance, virus was recovered on three separate occasions, all characterized by the occurrence of aphthous ulcers. On seven other occasions, no virus could be recovered, although two occasions represented instances of aphthous ulceration. Thus, herpes simplex virus was isolated only when aphthous ulcers were present but was not invariably recovered under these conditions. The two failures to recover virus are corroborative of the experience of others,[7] and it may be that optimum conditions are necessary for the recovery of virus inside the mouth. That the association of aphthous ulceration and herpes simplex virus is more than chance is supported by two further observations: 1. Inoculation of throat and mouth washings from 17 adults suffering from various febrile illnesses failed in all instances to produce herpetic disease in newborn mice. Seven of these patients were known to be subject to herpes of the mouth. 2. Provocation of labial herpes by surgical

section of the trigeminal nerve may be attended by concomitant typical aphthous ulcers of the buccal mucosa and palate.[8]

The probability of the multiple origin of aphthous ulcers must be considered and may explain the many failures in the past to implicate herpes simplex virus in their causation. Recently, Huebner and associates[9] have recovered a virus of the Coxsackie group from children with "herpangina," an illness characterized by ulcerative lesions of the pharynx.

COMMENT

Primary herpes simplex virus infection in the adult may be associated with disease of the mouth, throat, cornea, genitalia, skin, or central nervous system (Table 3) or with poorly defined illness with few localizing signs (Case 1). Stomatitis resembles the infantile disease and is accompanied with fever and malaise. Four serologically proved instances of primary adult herpetic stomatitis have appeared in the literature.[10] Gallardo[11] established the probable herpetic origin of certain cases of acute keratitis by the isolation of virus from corneal scrapings of five patients and demonstration of the appearance of neutralizing antibodies in

Papular lesions of the palate (Case 3).

convalescence in four patients from whom blood was obtained. Seventeen additional patients with herpetic keratitis mentioned in Gallardo's report were noted to possess neutralizing antibodies against herpes virus at the time of onset, and all had incurred previous attacks of cutaneous herpes. Although herpes simplex virus had been previously recovered from genital lesions, Slavin and Gavett[12] were the first to demonstrate primary genital infection by the virus. These workers recovered virus

8. Carton, C., and Kilbourne, E. D.: Predictable Provocation of Herpes Simplex by Trigeminal Posterior Root Section, to be published.

9. Huebner, R. J.; Cole, R. M.; Beeman, E. A.; Bell, J. A., and Peers, J. H.: Herpangina: Etiological Studies of a Specific Infectious Disease, J. A. M. A. **145**:628 (March 3) 1951.

10. (*a*) Scott, T. F. M.; Steigman, A. J., and Convey, J. H.: Acute Infectious Gingivostomatitis: Etiology, Epidemiology and Clinical Picture of a Common Disorder Caused by the Virus of Herpes Simplex, J. A. M. A. **117**:999 (Sept. 20) 1941. (*b*) Rogers, A. M.; Coriell, L. L.; Blank, H., and Scott, T. F. M.: Acute Herpetic Gingivostomatitis in the Adult, New England J. Med. **241**:330, 1949.

11. Gallardo, E.: Primary Herpes Simplex Keratitis, Arch. Ophth. **30**:217 (Aug.) 1943.

12. Slavin, H. B., and Gavett, E.: Primary Herpetic Vulvovaginitis, Proc. Soc. Exper. Biol. & Med. **63**:343, 1946.

from vaginal ulcers of three patients, and also from the pharynx of one suffering from a concomitant membranous pharyngitis. Ruchman and co-workers [13] recovered herpes simplex virus from cutaneous lesions of three adults and two children afflicted with Kaposi's varicelliform eruption and demonstrated a neutralizing antibody response against the virus in one of the adults. In an adult whose illness ended fatally, no neutralizing antibodies were ascertainable in acute phase serum. It is probable, therefore, that the latter patient also suffered a primary herpetic skin infection. Nagler has mentioned the development of neutralizing antibodies in two adults: In one instance no disease had been apparent [14]; in the other, infection may have been either inapparent or related to a pyogenic skin lesion of the leg.[15]

TABLE 3.—*Serologically Proved Primary Herpes Simplex Virus Infection of Adults (Published Data)*

Reference	Date	No. of Patients	Age, Yr.	Disease	Virus Recovered	Specific Antibody Response
Scott and others [10a]	1941	1	24	Stomatitis	+ Saliva	Neut.*
Armstrong [19]	1943	1	15	Meningitis	+ Spinal fluid	Neut.
Gallardo [11]	1943	4	..	Keratitis	+ Cornea	Neut.
			..	Keratitis	+ Cornea	Neut.
			..	Keratitis	+ Cornea	Neut.
			..	Keratitis	+ Cornea	Neut.
Nagler [14]	1944	1	..	None	0	Neut.
Slavin and Gavett [12]	1946	3	..	Vulvovaginitis	+ Vagina	Neut.
			..	Vulvovaginitis	+ Vagina	Neut.
			..	Vulvovaginitis and pharyngitis	+ Vagina and pharynx	Neut.
Nagler [15]	1946	1	..	? Inapparent; ? skin infection	0	Neut.
Ruchman and others [13]	1947	1	16	Varicelliform eruption	+ Skin lesions	Neut.
Kipping and Downie [22]	1948	1	37	Variola-like illness	+ Skin lesions	Neut.
Rogers and others [10b]	1949	3	21	Stomatitis	+ Saliva	Neut.
			34	Stomatitis	+ Saliva	Neut.
			..	Stomatitis	0	Neut.
Afzelius-Alm [20]	1950	6	40	Meningitis	+ Spinal fluid	C. F.†
			15	Encephalitis; vesicles of thumb	0	C. F.
			20	Genital herpes; bladder paresis; pleocytosis	0	C. F.
			27	Encephalitis	0	Neut.
			31	U. R. I.‡; headache; pleocytosis	0	Neut.
			31	U. R. I.; encephalitis; pleocytosis (8 mo.)	0	Neut. and C. F.
Kilbourne and Horsfall	1951	?	42	Fever; malaise; lymphadenopathy	+ Throat washing	Neut.
			21	Pharyngitis (without stomatitis)	+ Throat washing	Neut.

* Neut. indicates neutralizing antibody.
† C. F. indicates complement-fixing antibody.
‡ U. R. I. means infection of the upper respiratory tract.

Herpes simplex virus has long been postulated as a cause of nervous system disease of man [16] and has been variously endorsed as a cause of epidemic encephalitis [17] and cited as an "ubiquitous" virus recoverable on occasion from the

13. Ruchman, I.; Welsh, A. L., and Dodd, K.: Kaposi's Varicelliform Eruption: Isolation of the Virus of Herpes Simplex from the Cutaneous Lesions of Three Adults and One Infant, Arch. Dermat. & Syph. **56**:846 (Dec.) 1947.

14. Nagler, F. P. O.: A Specific Cutaneous Reaction in Persons Infected with the Virus of Herpes Simplex, J. Immunol. **48**:213, 1944.

15. Nagler, F. P. O.: A Herpes Skin Test Reagent from Amniotic Fluid, Australian J. Exper. Biol. & M. Sc. **24**:103, 1946.

16. Goodpasture, E. W.: Herpetic Infection, with Special Reference to Involvement of the Venous System, Medicine **8**:223, 1929.

17. Levaditi, C., and Harvier, P.: Le virus de l'encéphalite léthargique (encéphalite épidémique), Compt. rend. Soc. biol. **83**:354, 1920.

spinal fluid of asymptomatic persons.[18] It remained for Armstrong in 1943 [19] to adduce serological evidence that herpes simplex virus infection might be associated with a benign lymphocytic meningitis. The recent studies of Afzelius-Alm [20] have suggested the herpetic origin of six central nervous system infections of adults (Table 3). It should be noted that the implication of herpes simplex virus in fatal encephalitis [21] hinges on the evidence provided by virus recovery and/or pathological studies, so that the primacy of such infections remains unestablished. A bizarre illness characterized by severe constitutional symptoms and the subsequent appearance of skin lesions simulating smallpox has been described by Kipping and Downie [22] in a report which presents evidence of virus recovery and primary antibody response in the patient.

The first two cases described in the present communication add further evidence that primary herpes simplex virus infection of the adult may occur in association with clinical disease of variable nature distinctly different from the acute gingivostomatitis characteristic of infantile infections. It is notable that both patients were suspected of having infectious mononucleosis, and it may be conjectured that certain ill-defined illnesses now categorized clinically but not serologically as infectious mononucleosis may prove to be of herpetic origin.

The third case stands at variance with previous studies [7] in that herpes simplex virus was recovered on three occasions from recurrent aphthous ulcers. It is, therefore, likely that herpes simplex virus infection may account for some instances of recurrent aphthous ulceration and cannot be flatly rejected as an etiological agent of this process. Recovery of virus on the chorioallantoic membrane, as well as in the infant mouse, indicates that the present results are not dependent on the use of the latter host.

A corollary of the present investigation is the observation that, as in other diseases, varying neutralizing antibody concentrations against herpes virus may be found in different persons (Table 1) in contrast to the "all-or-nothing" character of the herpes antibody content of human serums postulated by others.[4b, c]

SUMMARY

Primary infection of the adult by the virus of herpes simplex may be attended by disease of protean nature, ranging from a mild stomatitis to acute inflammation of the brain and meninges.

Herpes simplex virus is recoverable on occasion from recurrent aphthous ulcers of the mouth and may be etiologically related to their pathogenesis.

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18. Flexner, S., and Amoss, H. L.: Contributions to the Pathology of Experimental Virus Encephalitis, J. Exper. Med. **41**:215, 1925.

19. Armstrong, C.: Herpes Simplex Virus Recovered from the Spinal Fluid of a Suspected Case of Lymphocytic Choriomeningitis, Pub. Health Rep. **58**:16, 1943.

20. Afzelius-Alm, L.: Aseptic Encephalomeningitides in Gothenburg, 1932-1948, Göteborg, Sweden, Elanders Boktryckeria Aktiebolag, 1950.

21. Smith, M. G.; Lennette, E. H., and Reames, H. R.: Isolation of the Virus of Herpes Simplex and the Demonstration of Intranuclear Inclusions in a Case of Acute Encephalitis, Am. J. Path. **17**:55, 1941. Zarafonetis, C. J. D.; Smadel, J. E.; Adams, J. W., and Haymaker, W.: Fatal Herpes Simplex Encephalitis in Man, ibid. **20**:429, 1944. Whitman, L.; Wall, M. J., and Warren, J.: Herpes Simplex Encephalitis, J. A. M. A. **131**:1408 (Aug. 24) 1946.

22. Kipping, R. H., and Downie, A. W.: Generalized Infection with the Virus of Herpes Simplex, Brit. M. J. **1**:247, 1948.

Case Reports

EFFECT OF CORTICOTROPHIN ON CHRONIC, SEVERE PRIMARY THROMBOCYTOPENIC PURPURA

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IDIOPATHIC thrombocytopenic purpura is one of the few disorders of obscure origin in which the effect of cortisone and corticotrophin (ACTH) therapy has not been extensively reported.[1] Results of administration of the hormones will be difficult to evaluate since spontaneous remissions are common. In some patients, however, the disease runs a chronic, unremitting course, so that the effect of agents of possible therapeutic value is of greater significance. During the past four years we have had the opportunity of observing the disease in a most severe and persistent form in a woman, and we wish to report the dramatic effect of corticotrophin administration.

REPORT OF CASE

The patient (A. M.) was a 41-year-old married school teacher who was well until 1945, when she noted the appearance of spontaneous bruising and small hemorrhages in the skin, along with bleeding of the gums and frequent nosebleeds. These symptoms continued without remission up to the time she entered Stanford University Hospital in December, 1947.

Her past and family history was not significant. She had had two normal pregnancies.

She was an intelligent, cooperative patient who was slightly overweight. There were many large ecchymoses over the body, and the lower legs and ankles were covered with petechial hemorrhages. The gums bled easily. There was no adenopathy, and the spleen was not palpable. The results of the remainder of the physical examination were normal.

There was no anemia. The leucocyte count was 6,000, with a normal differential count. Thrombocytes (platelets) were reduced in the blood smear, and repeated counts were all about 10,000 per cubic millimeter. Bone marrow aspiration showed a normal or increased number of megakaryocytes. The bleeding time was 12 minutes; the clotting time was normal, and clot retraction was absent.

Splenectomy was done on Dec. 27, 1947. The spleen weighed 206 gm. and was not unusual on histological examination.

There was a rise in thrombocyte count after operation to 200,000 per cubic millimeter. The bleeding time became normal, and the petechiae and other evidence of bleeding ceased for one month. There was then a return of all the previous symptoms and signs, with thrombocyte counts varying from 10,000 to 30,000. This state of affairs continued until 18 months after splenectomy, when hysterectomy was necessary to control the excessive vaginal bleeding at the time of menstruation. The thrombocyte count remained at the extremely low level after operation, and during the ensuing year varied from 10,000 to 30,000. Symptoms were constant.

In addition to the bleeding tendency, she noted chronic fatigue, with little variation. In August, 1950, she received corticotrophin, 25 mg. every four hours for three weeks, and then 25 mg. daily for two weeks. The Chart indicates the changes during the administration of

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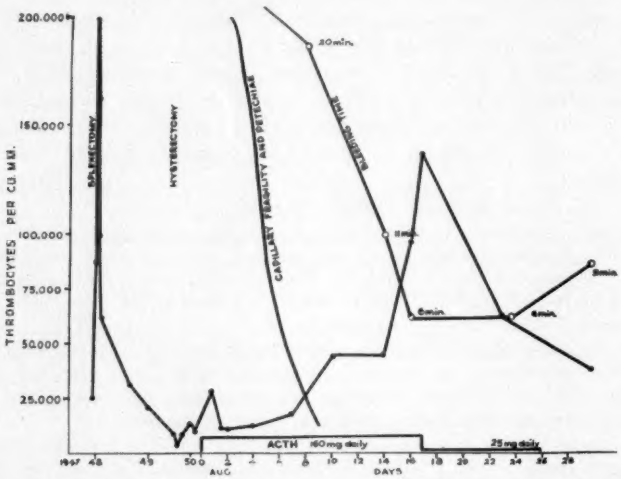
1. (*a*) Robson, H. N., and Duthie, J. J. R.: Capillary Resistance and Adrenocortical Activity, Brit. M. J. **2**:971 (Oct. 28) 1950. (*b*) Meyers, M. C.; Miller, S., and Bethell, F. H.: Administration of ACTH in Hypersplenic Syndromes, J. Lab. & Clin. Med. **36**:965 (Dec.) 1950.

corticotrophin. On the fourth and fifth day she noted a fading of petechial hemorrhages, even though she remained ambulatory. A significant rise in thrombocyte count was not present at the end of the first week, when spontaneous bleeding had ceased, but was demonstrable on the 10th day. Capillary fragility, as measured by the number of petechiae produced in 10 minutes on the forearm by 100 mm. of mercury pressure, was greatly increased and became normal about the same time that spontaneous bleeding ceased. The bleeding time as measured by both the Ivy and the Duke method approached infinity prior to administration of corticotrophin and fell to normal during the second week. The thrombocyte count rose to a high of 140,000 per cubic millimeter before the dosage of corticotrophin was reduced to 25 mg. daily. With the reduced dose the thrombocyte count dropped to 60,000, then 40,000. However, she continued to be symptom-free, and during the next two months thrombocyte counts were in the neighborhood of 50,000. Six weeks after discontinuation of corticotrophin therapy, she had several teeth extracted without abnormal bleeding.



Course of purpura in a patient with idiopathic thrombocytopenic purpura after corticotrophin administration. The capillary fragility and number of petechiae, which were both maximal, decreased to normal limits from the fourth to the eighth day. The bleeding time diminished from infinity to a normal level from the 8th to the 16th day during the time of the significant rise of the thrombocyte count.

The full-blown picture of thrombocytopenic purpura with which she was familiar returned gradually. On Dec. 5, 1950, she again exhibited easy bruising, numerous petechiae, and bleeding of the gums. The thrombocyte count was 22,000 per cubic millimeter. She was given 25 mg. of corticotrophin daily intramuscularly for two weeks. When this dosage failed to produce response, she was again admitted to the hospital for more intensive corticotrophin administration.

The response to the second course of corticotrophin therapy was somewhat sluggish as compared with that to the first course. Bleeding time, as determined by the Duke method, fell from more than 30 minutes to a normal range of 3 to 7 minutes after the fifth day. Spontaneous petechiae did not disappear until the 10th day. On the 16th day the thrombocyte count was 45,000, after a previous high of 19,000. Capillary fragility became normal, and the bleeding time, by the Ivy method, was normal for the first time on the 16th day. The dosage of corticotrophin was reduced to 150 and then to 75 mg. a day, but symptoms did not reappear. On the 29th day the thrombocyte count was 78,000.

She has received 25 mg. daily during alternate two week periods and has remained free of symptoms and signs of purpura for three months. The last thrombocyte count, done 60 days after the institution of therapy, was 110,000 per cubic millimeter. So far there has been no striking change in her appearance, and her general health is good.

COMMENT

The unequivocal clinical and hematological response that occurred twice in this patient indicates that a mechanism fundamental to the cause of primary thrombocytopenic purpura was modified by corticotrophin administration. It is of interest that the cessation of spontaneous purpura and the decrease in capillary fragility and bleeding time to normal preceded the rise in thrombocyte count. This sequence of events is not peculiar to the effect of corticotrophin or cortisone administration, since it has been observed frequently after splenectomy.[2]

Several explanations may be offered for the improvement in capillary fragility and bleeding time prior to the rise in thrombocyte count. The adrenal hormones may have a direct effect on capillary fragility, as suggested by Robson and Duthie.[1a] It is also possible that a toxic agent, perhaps immune in character, which is damaging for capillaries, is neutralized or its production suppressed by the hormones. A third explanation for the lag in rise of circulating thrombocytes may be the rapid utilization of the thrombocytes in the purpuric lesions. A rise in circulating thrombocytes may not be evident until the demand made by capillary lesions created by a lack of available thrombocytes is lessened.

The rise in thrombocyte count following corticotrophin administration may be explained by the postulate that primary thrombocytopenic purpura, like acquired hemolytic anemia and many other diseases known to respond to administration of the adrenal hormones, is due to abnormal activity of the immunological apparatus. Evidence has recently been presented that the low thrombocyte count is the result of an antithrombocyte antibody similar to that observed in acquired hemolytic anemia.[3] We[4] and others[5] have observed amelioration and, even, cessation of the abnormal hemolytic process during corticotrophin and cortisone administration. In most instances in our experience there is evidence of a fall in serum-antibody concentration and in some instances a diminution in the amount of adsorbed antibody. This suggests a suppression of production of the sensitizing protein, but other possibilities, such as interference with its activity as a sensitizing agent, may be important.

In view of other alternative postulates for the pathogenesis of primary thrombocytopenic purpura, the patient described here is particularly interesting, since splenectomy had been done with only temporary benefit and an accessory spleen was not found at the time of hysterectomy. It is obvious, therefore, that the spleen and accessory splenic tissue could not have been responsible for depressing the formation of thrombocytes or destroying thrombocytes by hypersequestration and

2. Robson, H. N.: Idiopathic Thrombocytopenic Purpura, Quart. J. Med. **18**:279 (Oct.) 1949.

3. Evans, R. S.; Takahashi, K.; Duane, R. T.; Payne, R., and Liu, C. K.: Primary Thrombocytopenic Purpura and Acquired Hemolytic Anemia: Evidence for a Common Etiology, A. M. A. Arch. Int. Med. **87**:48 (Jan.) 1951.

4. Evans, R. S.; Liu, C. K.; Duane, R. T., and Payne, R.: Unpublished data.

5. Dameshek, W.; Rosenthal, M. C., and Schwartz, L. I.: The Treatment of Acquired Hemolytic Anemia with Adrenocorticotrophic Hormone (ACTH), New England J. Med. **244**: 117 (Jan. 25) 1951.

phagocytosis. The continued activity of the disease in this patient was evidently due to the production of sufficient thrombocyte-destroying protein by tissues other than the spleen. An analogous situation is frequently observed in acquired hemolytic anemia after splenectomy.

The relapse on the cessation of corticotrophin administration indicates that the problems of continuous therapy will be present in the treatment of refractory primary thrombocytopenic purpura as in other conditions of obscure origin. In this patient the chronic thrombocytopenic purpura probably represents a continuous threat to her life from intracranial hemorrhage. Control with intermittent small injections will be attempted as long as possible. The intake of sodium chloride has been restricted, and she is taking 3.0 gm. of potassium chloride daily while receiving injections. So far no undesirable effects of hormone administration have been observed.

SUMMARY

A patient with unremitting, severe primary thrombocytopenic purpura following splenectomy was observed to have two distinct remissions in the disease after administration of corticotrophin (ACTH). The observed response fits in with the concept that the disease is due to abnormal activity of the immunological apparatus, similar to that demonstrated in acquired hemolytic anemia.

PAGET-SCHROETTER SYNDROME

Report of a Case of One of the More Unusual Neurovascular Syndromes of the Upper Limb

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THE PAGET-SCHROETTER syndrome is identified with the symptomatology of a neurovascular disease of the upper limb, the predominant lesion of which is obstruction of the axillary vein.

Among the many causes of shoulder pain the neurovascular syndromes occupy a unique and puzzling position. As the neural and vascular elements of the upper limb pass from the cervical and thoracic regions to converge and proceed into the axilla, they become vulnerable to minor trauma by the firmer supporting structures. Trauma by a cervical rib,[1] by a hypertrophied scalenus anticus muscle,[2] by a prevenous phrenic nerve,[3] by the lower border of the subclavius muscle and the costoclavicular ligament,[4] or by the clavicle and first rib[5] is a potentiality in 50 to 80% of adults.[6]

Read before the Section on Orthopedic Surgery of the New York Academy of Medicine, April 21, 1950.

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1. (*a*) Adson, A. N., and Coffey, J. R.: Cervical Rib: A Method of Anterior Approach for Relief of Symptoms by Division of the Scalenus Anticus, Ann. Surg. **85**:839, 1927. (*b*) Jones, F. W.: On the Relation of the Limb Plexuses to the Ribs and Vertebral Column, J. Anat. & Physiol. **44**:377, 1910. (*c*) Murphy, T.: Brachial Neuritis from Pressure of the First Rib, Australian M. J. **15**:582, 1910. (*d*) Todd, T. W.: The Relation of the Thoracic Operculum Considered in Reference to the Anatomy of Cervical Ribs of Surgical Importance, J. Anat. & Physiol. **45**:293, 1911. (*e*) Willshire, T.: Supernumerary First Rib, Lancet **2**:633, 1860. (*f*) Wood Jones, F.: Variation of the First Rib, Associated with Changes in the Constitution of the Brachial Plexus, J. Anat. & Physiol. **45**:249, 1911. (*g*) Blair, D. M.; Davis, F., and McKissock, W.: The Etiology of the Vascular Symptoms of Cervical Rib, Brit. J. Surg. **22**:406, 1935. (*h*) Stopfrod, J. S. B., and Telford, E. D.: Compression of the Lower Trunk of the Brachial Plexus by a First Dorsal Rib, ibid. **7**:168, 1919.

2. (*a*) Naffziger, H. C.: The Scalenus Syndrome, editorial, Surg., Gynec. & Obst. **64**:119, 1937. (*b*) Naffziger, H. C., and Grant, W. T.: Neuritis of Brachial Plexus Mechanical in Origin: Scalenus Syndrome, ibid. **67**:722, 1938. (*c*) Wilson, M. J.: Scalenus Anticus Syndrome, Bull. New York M. Coll. **4**:161, 1941.

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4. Lowenstein, P. S.: Thrombosis of the Axillary Vein: An Anatomic Study, J. A. M. A. **82**:854 (March 15) 1924.

5. Falconer, M. A., and Weddell, G.: Costoclavicular Compression of Subclavian Artery and Vein: Relation to Scalenus Anticus Syndrome, Lancet **2**:539, 1943.

6. (*a*) Falconer and Weddell.[5] (*b*) Wright, I. S.: The Neurovascular Syndrome Produced by Hyperabduction of the Arms, Am. Heart J. **29**:1, 1945; (*c*) Vascular Diseases in Clinical Practice, Chicago, The Year Book Publishers, Inc., 1948.

The vulnerable elements are the motor, sensory, and autonomic components of the brachial plexus, the axillary artery, and the axillary vein. They are subject to sudden, or continuous, or intermittent trauma by the firm portions of their dynamic chambers at the root of the upper limb. The symptoms of each neurovascular syndrome depend on which of these elements suffers the predominant trauma. The Paget-Schroetter syndrome is probably the best name which can at present be applied to the neurovascular syndrome in which injury to, and obstruction of, the axillary vein is predominant.[7]

It is apparently one of the least frequently seen or diagnosed of the syndromes, only somewhat more than 300 cases [3] having been reported since its first description by Sir James Paget in 1875.[8]

Characteristically, the syndrome occurs in a husky man in his third to fifth decade (Table 1). He presents a history of recently having "strained" his shoulder in the course of some unusual activity. It is almost always the dominant arm that is injured. By the time he comes to seek medical aid, the extremity is swollen

TABLE 1.—*Symptoms of Paget-Schroetter Syndrome*

I.	Shoulder pain, usually deltoid
II.	Palpable cord in axillary region
III.	Swelling of upper extremity
	A. Increased on effort or dependency
IV.	Distended superficial veins on the skin of the arm and pectoral region
V.	Discoloration of upper extremity
	A. Paleness, cyanotic mottling, or redness
	B. Increased on effort or dependency
VI.	Occurrence of majority of cases in the right arm of men
VII.	History of uncommon motion at the shoulder joint
VIII.	Absence of constitutional symptoms

from shoulder to finger tips, and he complains of sharp, burning pain in or about the shoulder or upper arm. He feels more comfortable when the limb is elevated, and when it is placed in a dependent position it becomes pale or cyanotic. The diagnosis is sometimes supported by the presence of a hard, tender palpable cord in the axilla along the course of the axillary vein. Constitutional symptoms are characteristically absent.[3] Disability is almost always prolonged.

CASE HISTORY

We had an unusual opportunity to study this syndrome when a 19-yr.-old, left-handed hospital employee presented himself with a complaint of severe pain in the deltoid region of the left shoulder. The pain had developed gradually during the previous 12 hours. Two days before, he had had some unusual exercise, pitching a softball. He did not recall any habitual sleeping posture, nor had he experienced any paresthesias previously. The cardiac status was normal. His past history was devoid of injury or disease of the limb. His father, however, had had a midthigh amputation on the left leg for Buerger's disease (thromboangiitis obliterans).

7. (*a*) Hughes.[3] (*b*) Lowenstein.[4] (*c*) Matas, R.: On the So-Called Primary Thrombosis of the Axillary Vein Caused by Strain: Report of a Case with Comments on Diagnosis, Pathogeny and Treatment of the Lesion in Its Medico-Legal Relations, Am. J. Surg. **24**:642, 1934. (*d*) Roelsen, E.: So-Called Traumatic Thrombosis of the Axillary and Subclavian Vein, Acta med. scandinav. **98**:589, 1939.

8. Paget, J.: Clinical Lectures and Essays, London, Longmans, Green & Co., 1875.

When seen, the patient carried his arm in a sling. He was moderately well developed, and no atrophies were apparent. On examination, the left arm from the deltoid region to the finger tips was noted to be extremely pale. Small distended veins were evident against the white background of his shoulder and pectoral region. However, the larger veins of the hand and forearm could not be observed or palpated because of the diffuse, tense swelling of the entire limb. The left forearm was 3.6 cm. larger in circumference. The edema, however, was not pitting. The skin was moist and cool, while that of the right arm was dry and warm. The left arm was tender to palpation from the finger tips to the deltoid region, and a hard, tender cord was palpable in the axilla.

Skin temperature, as measured by the thermocouple, on the ventral surface of the left forearm was 28 C. On the right forearm it was 35 C. Oscillometry was impossible because of the pain incurred by the inflated cuff. The radial pulse was, however, palpable. Determination of the patency of the ulnar arterial circulation by Allen's test [9] was also impossible because of the tense edema of the forearm and hand.

His oral temperature was 99.6 F., but a complete blood count, results of urinalysis, and sedimentation rate determination were within normal limits. Roentgenograms taken at this time revealed no abnormalities of the cervical vertebra or of the shoulder.

The patient was treated with rest, sedation, and elevation of the limb for three days, with no improvement. On the fourth day the pain was, if anything, severer. His oral temperature was 98.6 F. At this time we were impressed with the clinical evidence of arterial and venous spastic phenomena: the persistent pallor, edema, diaphoresis, and low skin temperature of the limb.[9b] There is no method other than therapeutic, that we know of, to distinguish vascular obstruction by spasm from vascular obstruction by thrombosis. However, DeBakey, Burch, and Ochsner [10] have demonstrated experimentally that on ligation of the femoral vein chemical irritation of the ligated segment causes a 52% decrease in oscillations over and above the loss of pulsations due to the ligation. They have also demonstrated that most of this loss due to vasospasm can be regained by interruption of the sympathetic reflex which begins in the adventitia of the irritated vein.

Thus, on the fourth day, using an anterior approach and 5 cc. of 1% procaine hydrochloride solution, a left stellate ganglion block was performed. The immediate success of the block was evidenced by ocular manifestations of Horner's syndrome. About 15 min. after the block, the patient reported relief from most of his pain and he was able to move his arm; the skin was dry and ruddy, and the skin temperature was 36 C. The temperature of the skin of the right arm was 35 C. The next day, the fifth day of illness, the patient reported his condition much improved, although some pain in the deltoid and axillary regions was present. A difference of only 1.5 cm. was noted in the circumferences of the left and right forearms. The stellate ganglion block was repeated. The sixth day, although some pain was still present, the skin was dry and the color was good. With the decrease in edema, the hard cord previously palpated with difficulty in the axilla became easily and distinctly palpable. A third stellate ganglion block was performed. It was followed by Horner's syndrome and an increase in skin temperature over that of the normal arm. The patient reported very little, if any, pain.

After two weeks, the shoulder pain again increased and the skin over the volar surface of the forearm became cooler and rather moist. Stellate ganglion block was repeated. Oscillometry could now be performed, and oscillometric and thermocouple readings were taken immediately before and immediately after this stellate ganglion block (Table 2). The thermocouple reading and the oscillometric index on the right forearm were 35 C. and 12, respectively. On the left arm the oscillometric index was 7 before block and 14 after block. The skin temperature was

9. (*a*) Wright.[9c] (*b*) Allen, E. V.; Barker, N. W., and Hines, E. A.: Peripheral Vascular Disease, Philadelphia, W. B. Saunders Company, 1946. (*c*) Allen's test for patency of the ulnar-artery system is performed by compressing the patient's radial artery at the wrist after he has squeezed the venous blood out of his hand by making a tight fist. If on extension of the fingers, the hand remains pale the ulnar arterial flow to the hand is obstructed.

10. DeBakey, M.; Burch, G. E., and Ochsner, A.: Effect of Chemical Irritation of Venous Segment on Peripheral Pulse Volume, Proc. Soc. Exper. Biol. & Med. **41**:585, 1939.

30 C. before block and 36 C. after block—conclusive evidence of the presence of vasospasm. Little, if any, evidence of arteriolar spasm is to be found in previously reported cases of this syndrome.

The following day a phlebogram was performed, with injection of 20 cc. of 40% iodopyracet (diodrast®) injection into a small vein on the ulnar aspect of the elbow. The phlebogram showed an unfilled segment of axillary vein which was interpreted as either a recanalizing thrombus or an area of segmental spasm.

In the next three months he presented himself at intervals varying from two days to four weeks with exacerbations of pain and decreased skin temperature which responded to stellate ganglion block. At the end of 10 weeks massage and active exercise were instituted. After four months, no axillary cord could be palpated and he was allowed full activity.

Follow-Up Examination.—Six months after onset the patient was reexamined (Table 3). Skin temperature and oscillometric readings were equal in both forearms in the anatomical position. However, when the left arm was put through a full range of motion with the

TABLE 2.—*Effects of Stellate Ganglion Block*

	Skin Temperature, Degrees	
	Right Arm	Left Arm
Before	35	30
After	35	36
	Oscillometric Index	
	Right Arm	Left Arm
Before	12	7
After	12	14

TABLE 3.—*Oscillometric Index in the Forearm During Motion at the Shoulder at Six Months' Follow-Up Examination*

Position	Right Arm	Left Arm
Anatomical	6	5.5
90-degree abduction	5	0
180-degree abduction	3	1
"Hyperabducted"	2.5	5

oscillometer attached, oscillations disappeared at 90-degree abduction with slight external rotation. A complaint of fatigue was made after 40 seconds in this position. Downward and backward bracing of the shoulders failed to cause significant loss of oscillations. Neurological examination revealed loss of light-touch and thermal distinctions in dermatomes of the fifth and sixth cervical nerves.

COMMENT

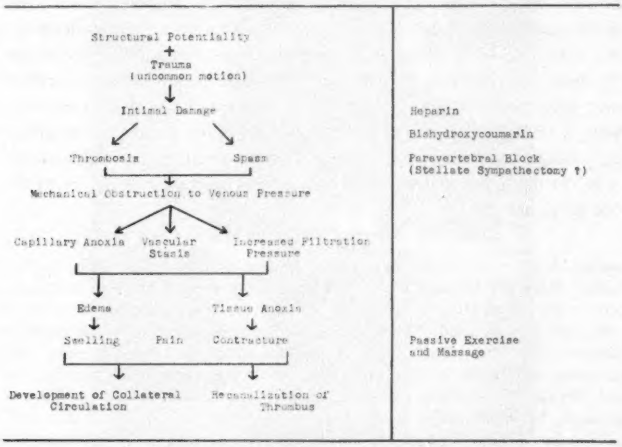
The case illustrates the usual course of events in the syndrome and presents several additional interesting features. The fact that oscillations were obliterated at 90-degree abduction with slight external rotation, even during periods of remission, reinforces our feeling that preexisting vulnerability of the neurovascular bundle exists in all these cases. On studying compression of the axillary vessels by the lower border of the subclavius muscle in 1927, Lowenstein [4] postulated this very position as the traumatic position.

As in most cases, a traumatic incident was present. The obstructed portion of vein was both palpable and demonstrable on phlebography. Diffuse arteriolar vasospasm made a surprisingly large contribution to the pathological condition of the limb, resulting in vascular stasis, capillary anoxia, increased filtration pressure, and

edema.[11] When vasospasm was eliminated by sympathetic block, circulation appeared adequate for the limb tissues. However, the thrombus, acting as an irritating focus, periodically set off the cyclic vasospastic reflex.[11b,d] This pathogenesis is represented schematically in the Figure.

It is our feeling that sympathetic block presents a feasible method of maintaining circulation of the upper limb until recanalization of the thrombus occurs. If, as has been postulated, there is a preliminary vasospastic stage of the disease before thrombosis occurs, sympathetic block plus the use of anticoagulants might abort the syndrome at this stage.

The instability of vascular pulsation in certain positions of this patient's arm plus the evidence of cervical-plexus injury indicates, as we have said, permanent crowding of the neurovascular bundle.



Sequence of events in Paget-Schroetter disease and suggested treatment at various stages. (Todd.[1d] Naffziger and Grant.[2b] Hughes.[3] Lowenstein.[4] Roelsen.[7d] DeBakey and others.[10] Ochsner and DeBakey.[11a-c] Smithwick.[11d] White, J. D., and Smithwick, R. H.: The Autonomic Nervous System, ed. 2, New York, The Macmillan Company, 1948.)

SUMMARY

A case of Paget-Schroetter syndrome is presented. The management of the case is detailed, and a pathogenesis is postulated.

ADDENDUM

Since this article was submitted for publication, there has been a recurrence of symptoms and the patient has undergone operation for excision of the subclavius muscle and tenotomy of the scalenus anticus. The postoperative course has been uneventful, and the patient appears to be fully recovered.

11. Ochsner, A., and DeBakey, M.: (*a*) Thrombophlebitis and Phlebothrombosis, C. Jeff Miller Lecture, South. Surgeon **8**:269, 1939; (*b*) Thrombophlebitis: The Role of Vasospasm in the Production of the Clinical Manifestations, J. A. M. A. **114**:117 (Jan. 13) 1940; (*c*) Therapy of Phlebothrombosis and Thrombophlebitis, Arch. Surg. **40**:208 (Feb.) 1940. (*d*) Smithwick, R. H.: Surgery of the Sympathetic Nervous System: The Role of Vasospasms in Acute Lesions Involving Major Peripheral Lesions, New England J. Med. **224**:329, 1941.

BILATERAL ULNAR NERVE PALSY AS A RESULT OF PRESSURE PHENOMENA

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AND
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ISOLATED peripheral-nerve paralysis is common and is most frequently caused by trauma. Multiple peripheral nerve lesions, particularly when symmetrical, are generally ascribed to the effects of some toxin or systemic disease, such as alcoholism, diabetes, or a demyelinating process or virus infection of the central nervous system. In the case described in this report, our initial impression of the ulnar nerve palsy, which appeared bilaterally and simultaneously, was that we were dealing with a cervical lesion, either in the spinal cord or in the vertebral column. However, roentgenograms of the elbows revealed peculiar bony deformities. Their discovery led to the belief that in some manner they were connected with the appearance of the paralysis.

REPORT OF A CASE

The patient, a 25-yr.-old white man, was admitted in September, 1950, to the Veterans Administration Hospital, Bronx, N. Y. His complaints were numbness, tingling, weakness, and atrophy in the distribution of the ulnar nerve, i. e., one and a half fingers (the fourth and fifth fingers) of each hand. These symptoms appeared suddenly in the upper extremities in late January, 1950; the right hand was involved more than the left. The patient found himself dropping his pencil or pen on writing and was clumsy in handling his drafting instruments. Because of the progressive nature of the complaints and his inability to continue his college work, he sought medical advice.

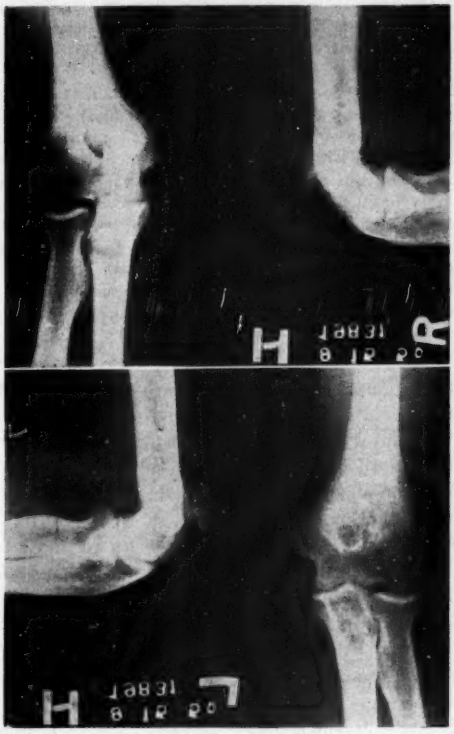
The patient was a sober, industrious, and healthy young man, who had recently completed six years of service in the United States Navy. Results of a general physical examination were noncontributory. Study of the upper extremities showed advanced atrophy of the adductor pollicis, the interossei, and the hypothenar eminence in each hand, but to a much less extent in the left. Motor strength was moderately diminished on the right side in the muscles supplied by the ulnar nerve, while muscle strength on the left was fairly well preserved. Both ulnar areas were hypesthetic. The threshold values for pain and touch were low. There seemed to be diminution of sweating in the ulnar-nerve distribution of the right hand. The patient was unable to lift his left arm to the vertical position, and there was some weakness, although not a marked atrophy, of the left triceps muscle. However, neurological examination did not show any abnormalities, except for suppression of abdominal reflexes. There was no pain on pressure on the spinous processes of the cervical portion of the spine, and neck motion was entirely free. There was no pain over the insertion of the scaleni muscles. Bracing of the shoulders and turning of the head to either side reduced the pulsation of the radial artery but did not obliterate it. A loss of the normal angular appearance of the ulnar side of the elbow joint on each side and about 10 degrees' limitation of complete extension were noted.

Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

Electrical reactions showed partial denervation of all small hand muscles supplied by the ulnar nerves; those in the arm were normal. The fibers of the abductor digiti quinti and the first interossei muscles showed the highest chronaxie values on both sides.

Electromyographic studies disclosed the highest degree of denervation, with the presence of spontaneous fibrillations in the right first interosseus. In the right abductor digiti quinti some signs of reinnervation were found, as manifested by complex potentials.

Roentgenographic examination of the elbows demonstrated ossicles posterior to the distal end of the humerus on a level with the supratrochlear notch or foramen. The symmetry in appearance and location led the radiologist to believe that these were patellae cubitorum.



Roentgenograms of right (top) and left (bottom) elbows. Note deformity of olecranon process and shape and position of ossicles over lower end of humerus. They are so similar that they can almost be superimposed.

The possibility that the fragments were old fractures or displaced epiphyses which had calcified was also entertained. Roentgenograms of the shoulders showed no osseous or articular abnormality.

The patient was questioned more closely about injuries in the remote past. These he had dismissed as being of no importance to his present condition. At the age of 12 yr., while sitting on a bicycle and leaning on a fence, he suddenly slipped and slid along the fence with his right elbow. The right elbow was swollen and painful for weeks. A roentgenogram was said to have shown "separation or fracture of the right olecranon." Three weeks later he fell from a chair and struck a table with his left elbow. Apparently, the same roentgenographic

appearance of the olecranon was seen on this side. Later roentgenograms revealed that the separation of the olecranon apophysis was still present. Although he had not had pain or muscular weakness since then, the patient was never able to elevate his arms completely.

Pointed questioning led us to another significant disclosure. Since September, 1949, at which time he had been discharged from the Navy, he had been attending college in an effort to complete his schooling. For at least eight hours a day and possibly more, he had been leaning on his elbows. This position is the customary one assumed by draftsmen and puts pressure for varying periods on structures at the bend of the elbow. The actual precipitating cause for the neuritis was felt to be on the basis of this change in occupation.

Exploration and transposition of the right ulnar nerve was undertaken. The nerve was so bound down by fine adhesions above and below the elbow that freeing the nerve from them was difficult. A fascial band had constricted the nerve in the lower half of the ulnar canal at the medial epicondyle. The nerve at this point showed marked thinning over a length of 1 to 1.5 cm.

Because the patient soon began to manifest improvement in sensation and to have better use of his right hand, operation was performed on the left ulnar nerve. Here, too, the trunk was found adherent to the surrounding structures in the ulnar groove; it was somewhat constricted distal to the groove. The nerve was freed and transplanted anteriorly.

The postoperative course was uneventful, there being rapid and striking improvement in both motor and sensory function. About six weeks after operation, the left hand was practically normal, except for slight diminution in sensation to touch in the fifth finger and along the outer aspect. On the right side, sensory function was gradually returning, but there was still weakness of the interossei and the adductor of the thumb.

Throughout this period the patient was treated by means of electrical stimulation and active use of his fingers and hands in physical and occupational therapy.

COMMENT

Injuries in the region of the elbow joint are prone to result in ulnar nerve palsies. Why these should appear bilaterally and simultaneously with such a long latent period between injury and appearance of paralysis is the chief point of this discussion. The trauma 13 years before had resulted in either a true fracture or slipping of the olecranon epiphyses. It is at about 12 years of age that these epiphyses become united to the shaft. The abnormal shortening of the olecranon process and the identical appearance of the accessory ossicles and their location in respect to the elbow joint are much more in favor of the latter possibility. Although the appearance and location resemble that of patellae cubitorum, which are congenital anomalies, the trauma and the inflammatory reactions that followed the original injuries are against such a diagnosis.

Regardless of the disputed origin of the bone fragments, it seems that the ulnar nerve, which is anatomically in a vulnerable position, was left even more unprotected than usual and that the fragments were displaced into a position over the ulnar grooves optimum for exerting pressure. One more link in the chain of coincidence was the change in occupation from naval duty to a type of student life which introduced pressure on the elbows for seven to eight hours a day. This, we feel, was the deciding factor in the production of the unusual bilateral and simultaneous paralysis of the ulnar nerves.

SUMMARY

An unusual case of bilateral ulnar nerve palsy secondary to pressure by displaced bone fragments is described.

Progress in Internal Medicine

STUDIES IN MITRAL STENOSIS

I. A Correlation of Physiologic and Clinical Findings

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MITRAL stenosis is unique in that it is the only type of heart disease in which there is a mechanical barrier to the entrance of blood into the left side of the heart. This results in a tendency to congestion and elevation of pressure throughout the pulmonary circulation. Unlike the dynamic situation in left ventricular failure, this stenotic dam may remain static for a long period, with little change in the clinical state of the patient. Ultimately, however, secondary effects on the left auricle, the pulmonary circulation, or the right ventricle develop to such a degree as to determine the final outcome. This condition lends itself, therefore, to the theoretical possibility of relief by reconstructive surgical measures at the site of obstruction, the mitral valve. In recent years this theoretical possibility has been increasingly translated into the realm of the practical with the development of operative procedures which are giving substantial relief to patients without a prohibitive operative mortality.[1] It is, therefore, desirable to review the whole

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(Footnote continued on next page)

subject of this disease state—its pathologic aspects, the nature of the disturbance in circulatory physiology, and the entire clinical life history of the disease. The place of surgery in this condition can thus be evaluated, as regards both the various factors determining the selection of cases for operation and the prognostic implications.

Other communications in this series deal with clinicopathologic correlations,[2] with the surgical aspects of the operative procedure and its results,[1g] and with the selection of patients for operation.[1h] The present paper is a report of the findings in regard to the disturbance in circulatory physiology which occurs and its relation to clinical symptoms and signs. A preliminary report on some of this work has already been made.[1b]

The study is particularly concerned with the cardiac output at rest and during excerise and pressure relations in the pulmonary circulation in correlation with the clinical condition of the patient.

PROCEDURES

The patients selected were studied by means of the usual routine clinical tests, including teleroentgenogram, electrocardiogram, and determination of vital capacity. Cardiac catheterization was carried out as previously described,[3] and measurements were made at rest and during mild exercise. Blood flows were calculated by the Fick method, with oxygen analysis on arterial and mixed venous blood performed by the method of Van Slyke, and oxygen consumption was calculated from expired air. The carbon dioxide content of the expired air was determined by the Haldane method and the oxygen content by either the Haldane technique or a Pauling oxygen analyzer. Pulmonary "capillary,"[4] pulmonary artery, right ventricular, and right auricular pressures were measured in most instances by a Sanborn electromanometer. In a few cases Hamilton manometers were employed. The resistances to blood flow in the lungs were calculated as follows:

$$\text{Pulmonary arteriolar resistance} = \frac{PA - PC}{CO} \times 1{,}332 \text{ dynes sec. cm.}^{-5}$$

$$\text{Total pulmonary resistance} = \frac{PA}{CO} \times 1{,}332 \text{ dynes sec. cm.}^{-5}$$

where PA indicates mean pulmonary artery pressure in millimeters Hg; PC, mean pulmonary "capillary" pressure in millimeters Hg; CO, cardiac output in cubic centimeters per second; 1,332, conversion factor from millimeters Hg to dynes seconds cm.$^{-5}$

The subjects were studied in either the postabsorptive state or about three hours after a light meal (fruit juice, milk, and toast). Exercise was carried out in the supine position by

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Circulation **1**:329 (March) 1950. (*e*) Glover, R. P.; Bailey, C. P., and O'Neill, T. J. E.: Surgery of Stenotic Valvular Disease of the Heart, J. A. M. A. **144**:1049 (Nov. 25) 1950. (*f*) Baker, C.; Brock, R. C., and Campbell, M.: Valvulotomy for Mitral Stenosis: Report of 6 Successful Cases, Brit. M. J. **1**:1283 (June 3) 1950. (*g*) Harken, D. E.; Dexter, L.; Ellis, L. B.; Farrand, R. E., and Dickson, J. F.: The Surgery of Mitral Stenosis: III. The Technique of Finger Fracture Valvuloplasty, Ann. Surg., to be published. (*h*) Harken, D. E.; Ellis, L. B.; Dexter, L.; Farrand, R. E., and Dickson, J. F.: The Responsibility of the Physician in the Selection of Patients for the Surgical Treatment of Mitral Stenosis, Circulation, to be published.

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pushing against weighted pedals. After one-half to one minute of preliminary exercise, expired air was collected for a two-minute period, and blood samples were collected at the end of one minute of the sampling period; pressure measurements were made immediately at the end of exercise and were taken at intervals until the control level was reached.

MATERIAL

Thirty-eight patients with mitral stenosis, 19 men and 19 women, were studied. The ages of the patients ranged from 20 to 58 yr.; all but four were between 26 and 46. There was no age or sex correlation with the degree of cardiac disability. The youngest and oldest were in the group with the mildest symptoms. In two, catheterization was performed twice prior to operation (mitral valvuloplasty), and in one, four times. Postoperative studies will be reported separately. In 14 of the patients the presence of an apical systolic murmur of Grade 2 or more [5] suggested some degree of mitral insufficiency. None had evidence of marked insufficiency, such as left ventricular enlargement or, when studied, the typical "insufficiency" tracing in the pulmonary capillary pressure curve.[6]

In none was there unequivocal evidence of organic involvement of any other valve. An aortic systolic murmur and electrocardiographic evidence of left ventricular enlargement in Patient 8 made the presence of an aortic-valve lesion probable. In 10 patients a Grade 1 to 2 early blowing diastolic murmur was present at the left border of the sternum near the third interspace but was not heard to the right of the sternum. In a few of these patients it was impossible to be sure that this murmur was different from a widely transmitted mitral diastolic murmur. This "aortic" murmur in some of the patients may have been produced by slight aortic regurgitation, but none had peripheral signs, wide pulse pressure, or evidence of left ventricular hypertrophy. Furthermore, of the three patients with "aortic" murmur who subsequently were observed at autopsy, two had normal aortic valves, and the third showed only slight thickening of the aortic-valve cups. All these patients had greatly enlarged pulmonary arteries. It is likely, therefore, that many of these "aortic" murmurs were due to functional pulmonic regurgitation (Graham Steell murmur).

Tricuspid insufficiency was demonstrated clinically and by right auricular pressure curves [7] in three patients. At autopsy in one patient this insufficiency proved to be functional. No patient had any clinical evidence of active rheumatic infection or of any other type of heart disease. However, in two active rheumatic myocarditis was observed at post-mortem examination, and in two others biopsy specimens of the left auricular appendage showed Aschoff bodies, although post-mortem study of the rest of the heart within a few days disclosed no further evidence of rheumatic activity.

Sixteen of the 38 patients had auricular fibrillation at the time the studies were made. In three auricular fibrillation developed later. One patient was studied when he had sinus rhythm, and again after he went into auricular fibrillation; another had previously had auricular fibrillation but was in sinus rhythm at the time of investigation.

The patients were graded Class 1 to 4 on the basis of their degree of incapacity from chronic dyspnea and other pulmonary symptoms. This classification, in general, agreed with the criteria of classification of the American Heart Association, except that the patients in Class 1 had dyspnea on marked exertion. Only one (Patient 1) had no dyspnea on effort. In addition, a subgroup of 11 patients subject to attacks of acute pulmonary edema was separated out, since their clinical course was somewhat different from that of the group as a whole. The patients in this subgroup experienced varying degrees of chronic dyspnea. The grading was arbitrary because of the protean clinical pictures and the large subjective element in many of the symptoms.

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5. Severity of murmur was based on a scale of gradation from 1 to 6.

6. Gorlin, R.; Haynes, F. W.; Goodale, W. T.; Sawyer, C. G.; Dow, J. W., and Dexter, L.: Studies of the Circulatory Dynamics in Mitral Stenosis, Am. Heart J. **41**:10 (Jan.) 1951.

7. Bloomfield, R. A.; Lauson, H. D.; Cournand, A.; Breed, E. S., and Richards, D. W., Jr.: Recording of Right Heart Pressures in Normal Subjects and in Patients with Chronic Pulmonary Disease and Various Types of Cardio-Circulatory Disease, J. Clin. Invest. **25**:639 (July) 1946.

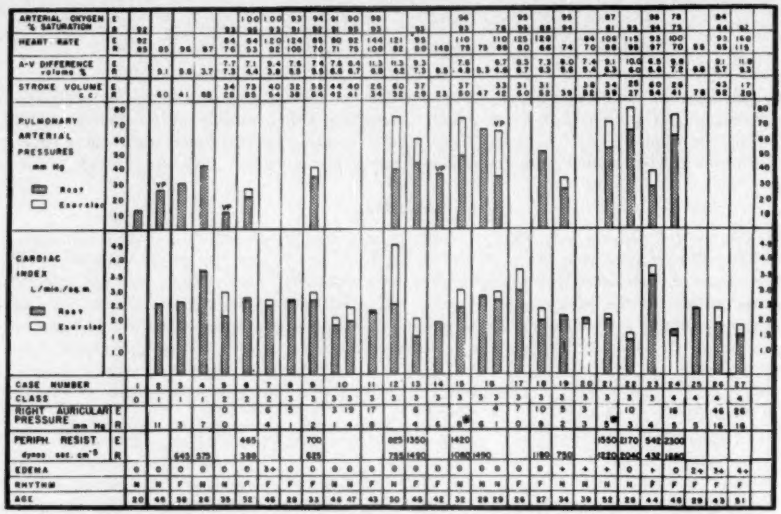

Chart 1.—Data on patients with mitral stenosis not subject to acute pulmonary edema. Asterisk indicates venous pressure. *VP* indicates mean right ventricular pressures (one-half the pulse pressure plus diastolic pressure). *R* indicates rest; *E*, exercise; *N*, normal sinus rhythm, and *F*, auricular fibrillation.

ARTERIAL OXYGEN % SATURATION	E						92			93																		
	R	93	88			91		99	97	92			95			90	85	89										
HEART RATE	E	103				85	94	100	136							152		120	105									
	R	64	90	122		50	82	88	81	82		94	97	152	75	100	88											
A-V DIFFERENCE volume %	E	7.4				7.1	5.5	4.6	11.5			7.0	4.8	3.8		6.2	5.4											
	R	4.4		3.9		4.2	3.8	4.3	7.0	4.4		6.6	5.2	3.4	9.0	5.0	5.7											
STROKE VOLUME c.c.	E	39				56	63	78	30			86	62	27		50	50											
	R	31		34		93	62	60	51	85		52	46	30	30	48	38											
PULMONARY ARTERIAL PRESSURES mm. Hg (Rest / Exercise)		80 70 60 50 40 30 20 10																										
CARDIAC INDEX L/min./sq. m. (Rest / Exercise)		5.0 4.0 3.0 2.0 1.0																										
CASE NUMBER		28	29	30	31	32	33	34	35						36	37	38											
CLASS		1	2	2	3	3	3	3	3	3	3	3	4	4	4	4												
RIGHT AURICULAR PRESSURE mm Hg	E				3			10				6	25			+2	-4											
	R		9*	3	2	2	2	0	3	7	0	19	20*			-1	-4											
PERIPHERAL RESISTANCE dynes sec. cm⁻⁵	E	392				750	544			690	745				2550													
	R	306		862		465	590		342	965	800	1415																
EDEMA		0	0	0	0	0	0	±	0	0	0	2+	+	0	+													
RHYTHM		N	N	N	N	N	N	N	N	N	N	F	F	N	N													
AGE		35	33	30	23	30	28	28	26 — 28						37	36	46											

Chart 2.—Data on patients with mitral stenosis subject to acute pulmonary edema. See legend to Chart 1 for explanation of symbols.

A striking finding was the lack of any close correlation between the vital capacity, measured at rest, and the severity of pulmonary symptoms. Seven of 14 patients with Class 3 disability had a vital capacity in excess of 3,000 cc. In a few cases the vital capacity was determined at the end of exercise. It either showed no change or was reduced by a few hundred cubic centimeters.

In all cases electrocardiograms were taken, but in only 30 were six or more precordial lead V's and unipolar limb leads taken. All these showed an electrically vertical heart, with the exception of one case in which the position was indeterminate and one case in which the heart was horizontal. In 24 the electrical axis was deviated to the right; in 12 it was normal, and in two it was deviated to the left. In five patients (one in Class 1; two in Class 2; two in Class 3) there were no electrocardiographic findings suggesting enlargement of either ventricle. In 18 evidence of right ventricular hypertrophy was present, and an additional nine showed incomplete right bundle branch block in leads CV_1 or CV_5, making a total of 27 who presumably had right ventricular hypertrophy. In one of the 27 patients and in another without clear evidence of right ventricular hypertrophy, there was electrocardiographic evidence consistent with left ventricular enlargement. The first of these was the patient already mentioned with a systolic murmur in the aortic area. The second was a man aged 42, who had a diastolic but no systolic murmur at the apex and an equivocal "aortic" diastolic murmur in the third interspace near the sternum. In this patient, although six precordial lead V's were taken, unipolar limb leads were not available. In the remaining patients, it was impossible to state whether ventricular hypertrophy existed because an insufficient number of leads was taken.

Seventeen of these patients subsequently died, and post-mortem examinations were performed in 12.

In the patients examined post mortem, the degree of stenosis of the mitral valve was graded from 1 to 3. In Grade 1 stenosis the valve was ordinarily described as "it would admit two fingers." No patients with this degree of stenosis came to post-mortem examination. In Grade 2 stenosis the valve was usually described as "admitting one finger." Grade 3 stenosis was the most marked, and the valve was usually described in terms of "will not admit the tip of a finger," "extreme fish-mouth stenosis, etc."

The descriptive classification by Parker and Weiss [8] of the degree of structural changes in the lungs was followed. These have also been described by Larrabee, Parker, and Edwards [9] and will be discussed by us in detail in our communication on pathologic correlations in this disease.[2]

RESULTS

Cardiac Output.—The normal cardiac index at rest averages 3.3 l. per minute per square meter of body surface and rarely falls below 2.7 l. The cardiac output at rest was measured in 35 patients in this study, and in eight it was found to be 3.0 l. or slightly higher; in nine it was between 2.7 and 3.0 l., and in the remaining 17 it fell below 2.7 l.

The response of normal persons to mild or moderate excercise is an increase in minute volume output, roughly parallel to, but somewhat less than, the increase in oxygen consumption.[10] Twenty-eight patients were given an exercise which

8. Parker, F., Jr., and Weiss, S.: The Nature and Significance of the Structural Changes in the Lungs in Mitral Stenosis, Am. J. Path. **12**:573 (Sept.) 1936.

9. Larrabee, W. F.; Parker, R. L., and Edwards, J. E.: Pathology of Intrapulmonary Arteries and Arterioles in Mitral Stenosis, Proc. Staff Meet., Mayo Clin. **24**:316 (June 8) 1949.

10. (*a*) Bock, A. V.; Vancaulaert, C.; Dill, D. B.; Folling, A., and Hurxthal, I. M.: Studies in Muscular Activity: III. Dynamical Changes Occurring in Man at Work, J. Physiol. **66**:136 (Oct.) 1928. (*b*) Hickman, J. B., and Cargill, W. H.: Effect of Exercise on Cardiac Output and Pulmonary Arterial Pressure in Normal Persons and in Patients with Cardiovascular Disease and Pulmonary Emphysema, J. Clin. Invest. **27**:10 (Jan.) 1948. (*c*) Riley,

(*Footnote continued on next page*)

increased their oxygen consumption by 50 to 100% over the resting level. In five the cardiac index increased by 1 l. or more; in 13 the increase was less than 1 l.; in eight there was virtually no change, and in two the cardiac index decreased. Although the exercise was mild, it was sufficient to produce obvious dyspnea in all patients of Class 2 or more, and in several there was coughing. In one patient (29) with associated asthmatic bronchitis, exercise resulted in a sense of suffocation and severe coughing and led to the termination of the experiment. In no other patients was the clinical picture of pulmonary edema produced, although the lungs were not examined for rales at the end of the exercise.

Some indication of the cardiac output is given by the arteriovenous oxygen difference. This difference in oxygen content between arterial and venous blood represents the peripheral extraction and utilization of oxygen. It is inversely proportional to the blood flow. The normal resting arteriovenous difference averages 4.0 to 4.5 vol. % and is rarely greater than 6.0. In this group of patients, 15 had

Pulmonary Capillary Pressures and Pulmonary Artery Resistances

Patient No.	Class	Mean Pulmonary Artery Pressure, Mm. Hg	Pulmonary "Capillary" Pressure, Mm. Hg	PA-PC Differential, Mm. Hg	Pulmonary Arteriolar Resistance, Dynes Sec. Cm.$^{-5}$	Comment
Average normal	..	15	10	5	100	Resting
1	0	15	10.5	4.5	...	Resting
6	2	22	13	9	159	Resting
	..	27	17	10	175	Exercise
29	2	37	19	18	...	Resting
13	3	43	18	25	870	Resting
	..	60	30	30	670	Exercise
22	3	65	41	24	740	Resting
35 (2d study)	3	41	33	8	188	Resting
	..	70	52	18	178	Exercise
35 (4th study)	4	64	34	30	670	Resting

a resting arteriovenous difference greater than 6.0 vol. % and in only 10 was it less than 5.0.

Normally during exercise the greater minute volume output is accomplished by an increase both of the stroke output and in the heart rate. In this study only ten of the patients showed an increase in stroke output with exercise, and in seven the increase was less than 10 cc., with an increase of only 1 or 2 cc. in three of these.

A disproportionate increase in heart rate with the mild exercise was also observed in many of these patients. On 18 occasions the heart rate was 100 beats per minute or more during the exercise, in three instances ranging from 140 to 160.

Pulmonary Artery Pressure.—The normal mean pulmonary artery pressure at rest averages 15 mm. of mercury. This measurement was made in 22 patients, and the right ventricular pressure was obtained in three more. In only two was

R. L.; Himmelstein, A.; Motley, H. L.; Weiner, H. M., and Cournand, A.: Studies of the Pulmonary Circulation at Rest and During Exercise in Normal Individuals and in Patients with Chronic Pulmonary Disease, Am. J. Physiol. **152**:372 (Feb.) 1948. (*d*) Dexter, L.; Whittenberger, J. L.; Haynes, F. W.; Goodale, W. T.; Gorlin, R., and Sawyer, C. G.: Effect of Exercise on Circulatory Dynamics of Normal Individuals, J. Appl. Physiol. **3**:439 (Feb.) 1951.

the resting mean pulmonary pressure under 20 mm. of mercury, and in one additional patient, in whom the right ventricular pressure only was obtained, it was normal. On mild exercise persons with normal circulation respond with little or no increase in pulmonary artery pressure.[10b-d] In the entire group of patients with mitral stenosis in whom the response was studied, a definite increase, often of marked degree, was noted during exercise.

Pulmonary "Capillary" Pressure (Table).—The normal pulmonary "capillary" pressure is about 10 mm. of mercury.[4] It was determined seven times in six patients of this group and was found to be elevated in all but one.

Arterial Oxygen Saturation.—In only six patients was the resting arterial oxygen saturation normal, that is, 95% or higher. In 16 it fell between 90 and 95%, and in eight it was below 90%. All but one of the patients with a saturation below 90% had severe disability (Class 3 or 4), although the converse did not hold; i. e., many patients with marked pulmonary disability had normal or but slightly reduced oxygen saturation. During exercise the oxygen saturation remained unchanged or improved in all but one instance.

CORRELATIONS AND INTERPRETATIONS

A correlation between the degree of pulmonary disability and the reduction in the cardiac output held only in a rough way. The one patient (28) with mild disability only in whom exercise studies were done showed a normal increase with exercise, and, in two of the four patients in this group in whom the cardiac output was measured, the resting cardiac index was above 3 l. Class 2 and 3 patients showed great variation in the output findings. All the patients with Class 4 disability had unusually low cardiac outputs both at rest and during exercise, with the exception of two who were subject to attacks of acute pulmonary edema and in whom the cardiac index during exercise exceeded 3 l. This group of patients who were subject to attacks of acute pulmonary edema proved to be particularly interesting. All but one were in sinus rhythm. Only two of the group failed to have a cardiac index of 3.0 l. or more after exercise (in one of them exercise was not done, but his resting index was only 1.7 l.), and in several of them response to exercise was quite normal. This was in distinct contrast to patients with an equivalent degree of chronic pulmonary difficulty, who, for the most part, had reduced cardiac outputs. This group of patients subject to acute pulmonary edema will receive special consideration later.

All but one of the patients with mild disability were in sinus rhythm, in contrast to only two of the eight with severe dyspnea. The others in these groups had auricular fibrillation. Essentially the same number of patients in the intermediate groups (Class 2 and 3) had sinus rhythm and auricular fibrillation. There were so many factors that might influence blood flow and so much variability in the output findings that this study throws no light on the influence of heart rhythm per se on the output. It was observed that many of the patients had a tendency toward disproportionate increase in heart rate with exercise, but there was no correlation between this and the presence of auricular fibrillation or a tendency toward attacks of pulmonary edema.

A definite correlation was noted between the severity of pulmonary symptoms and the level of the pulmonary artery pressure. This was true at rest and was even more striking during exercise. However, it must be pointed out that three

of the five patients with mild disability had resting mean pressures that were as high as those shown by many patients with a greater degree of disability.

Pulmonary arteriolar resistance was measured in four patients (Table). In one of these (6) the resistance was only slightly elevated and did not change materially with exercise, and this corresponded with the comparatively good clinical condition of the patient. In two (Patients 13 and 22) resistance was markedly elevated, and in the first of these it appeared to be slightly diminished during exercise. Both these patients were seriously disabled by chronic dyspnea but were not subject to attacks of acute pulmonary edema. In the fourth patient (35) the pulmonary arteriolar resistance was determined twice; the first time it was only slightly elevated, and the second time it was markedly increased. Further discussion of this patient will be made later.

The total pulmonary resistance was estimated in 20 patients and was increased in all above the normal of 200 to 300 dynes seconds cm.,$^{-5}$ although in two (Patients 6 and 28) this increase was slight. In a third (35) already mentioned, the total pulmonary resistance was determined on four occasions and showed a progressive tendency to become elevated concomitantly with increase in the patient's degree of chronic disability.

Peripheral Edema and Right-Sided Failure.—All the patients subject to edema had had it in some degree within a few days of the catheterization studies. All required a low-sodium diet and diuretic measures to prevent accumulation of fluid. However, in all but three the edema was minimal on the day of study. These three exceptions had well-marked edema and clinical evidence suggesting tricuspid insufficiency.

Seven patients had definitely abnormal right auricular pressures, with either a resting level of 10 mm. of mercury or higher (in one patient with an elevated venous pressure, right auricular pressures were not measured) or a marked increase on mild exercise to levels above 16 mm. of mercury. In six more there was a borderline elevation in the pressure, although in two of these patients the findings were equivocal. The resting pressure in one of them was 5 mm. and was not determined after exercise, and in the other it was 4.5 mm., rising to 6.0 mm. during exercise. Both of these patients were edematous. The three who had the most notable elevation of pressure had clinical evidence of tricuspid insufficiency, with a decided tendency toward edema, pulsating jugular veins, and enlarged, pulsating livers. In four patients who showed definitely abnormal auricular pressures and in two more with borderline findings, there had never been any edema, although two of the patients did have enlargement of the liver by palpation. Four patients in whom peripheral edema easily developed had normal right auricular pressure, although in one the auricular pressure was not measured during exercise. An additional patient had normal venous pressures on repeated tests.

For the most part, patients with abnormally high auricular pressure had low cardiac output. In only one instance was the cardiac index at rest or after exercise more than 2.4 l., and that was 2.7 l. at rest. The patients with suggestively abnormal auricular pressure response had cardiac indexes after exercise ranging from 2.4 to 2.8 l. Of the five patients with edema but with normal auricular or venous pressure, three had low cardiac output (cardiac index, 1.9 to 2.2 l.), but in two the output was well maintained after exercise (cardiac index, 3.5 and 3.8 l.). A number

of patients who had normal auricular pressure responses and had never had edema were noted to have cardiac indexes after exercise as low as those observed for the edematous patients.

Correlations of Autopsy Observations, Clinical Course, and Physiologic Measurements (Chart 3).—Of the group in whom physiologic measurements were made, 17 subsequently died and in 12 post-mortem examinations were carried out. From an additional patient, who died at the time of operation on the mitral valve, a biopsy specimen of the lungs was obtained. Of this group of 13 patients, 10 died after operation, within a few days of the time that the cardiac catheterization measurements were made. In another patient (29) who died after operation, careful clinical observations had been recently made, but about 11 mo. after the cardiac catheterization measurements. The clinical course did not suggest that there had been a marked

ACTIVE RHEUMATIC CHANGES		0	0	+	Biopsy + P.M. 0	Biopsy + P.M. 0	0	0	0	+		0	
ORGANIC PULMONARY DISEASE	3+	3+	2+	1+	1+	3+	3+	2+	3+	3+	2+	3+	3+*
MITRAL STENOSIS	3+	3+	3+	3+	3+	3+	2+	3+	3+	3+	2+	3+	
PULMONARY ARTERIAL PRESSURE mm. Hg (75, 50, 25) Rest / Exercise													
CARDIAC INDEX L./Min./Sq. m. (4.0, 3.0, 2.0, 1.0) Rest / Exercise													
DYSPNEA	1+	3+	3+	3+	3+	3+	4+	4+	2+	3+	3+	4+	4+
ACUTE PULMONARY EDEMA	0	0	0	0	0	0	0	0	2+	3+	2+	3+	3+
EDEMA	0	0	0	1+	1+	0	2+	4+	0	0	±	1+	0
PATIENT	4	10	12	20	21	22	25	27	29	33	34	36	37

Chart 3.—Correlation of clinicophysiologic and post-mortem findings. Asterisk indicates biopsy of lung was done, but no post-mortem examination was made.

exacerbation of her disease in the interval since the measurements. This patient was one whose course was marked by a series of episodes of acute pulmonary edema associated with acute pulmonary infections, between which the cardiac disability was moderate. The two remaining patients on whom post-mortem examinations were carried out were Patients 25 and 33, who died about six months following physiologic study. The first of these died, in chronic congestive heart failure and the second during an acute attack of pulmonary edema. All the patients in this group of 13 were in Class 3 or 4, so far as their chronic pulmonary disability was concerned, with the exception of two: one, Patient 29, who was just mentioned, and the other, Patient 4, who had dyspnea only on great exertion and had had no attacks of pulmonary edema but had suffered several episodes of severe and almost exsanguinating pulmonary hemorrhage.

In patients with advanced pulmonary arterial disease and organic changes in the alveolar walls, there was no consistent clinical pattern. One patient in whom such changes were observed (4) had had almost no symptoms except recurrent bouts of hemoptysis and was able to play tennis. Another patient (25), whose lungs showed as marked changes of this type as have been observed in this laboratory, had a steady downhill course over a period of years, manifested not only by progressive dyspnea but even more by right-sided failure and peripheral congestion. Although he had had one or two episodes of hemoptysis, he had never had pulmonary edema. On the other hand, there were four patients whose chief disability was due to repeated attacks of pulmonary edema and who showed advanced (Grade 3) pulmonary vascular changes and interalveolar lesions. The only other patient in this group who suffered from acute attacks of pulmonary edema had moderately severe organic changes in the lungs.

Although most of the patients in the group tended to have both severe stenosis and high-grade pulmonary changes, beyond this there was no good correlation between the presence of such organic changes in the pulmonary circulation and lungs and the degree of stenosis in the mitral valve. Patient 24, already mentioned, who had extreme changes in his pulmonary blood vessels, had only Grade 2 stenosis of the mitral valve. There were two patients with marked stenosis (Grade 3) of the mitral valve who showed minor organic changes in the pulmonary circulation, and one further patient with extreme stenosis of the mitral valve who showed only moderate (Grade 2) alterations in the pulmonary circulation. The lack of a relationship between the presence of organic pulmonary changes and the degree of mitral stenosis has been observed by us in a larger series reported on elsewhere.[2]

That extreme mitral stenosis can exist without marked chronic dyspnea is illustrated by Patients 4 and 29 and has been commented upon by us in another communication.[2]

The cardiac index at rest and even after exercise, although usually reduced below the average normal at rest of 3.0 l. per minute per square meter of body surface, showed no constant relation to the symptoms, the degree of stenosis, or the lung changes. Note, for example, the well-maintained outputs in Patients 4, 20, 33, and 39, although each of them had serious mitral stenosis and three of the four had advanced organic changes in the pulmonary circulation.

There was a much better correlation between the pulmonary artery pressure and the degree of disability, as we have discussed previously, although the observations on pulmonary artery pressure in this series were too few to be of any great significance. However, there was no good correlation between the pulmonary artery pressure and the degree of mitral stenosis, or the presence of organic pulmonary changes, or a tendency to attacks of acute pulmonary edema. The total pulmonary resistances (Charts 1 and 2) were measured in six patients, each of whom had severe mitral stenosis. The resistances were elevated in all but varied greatly, from 575 to 2,550 dynes seconds $cm.^{-5}$ This correspondence between organic vascular changes in the lungs and the resistances was not close, although the two patients (22 and 36) with the highest resistances also had the most pronounced structural changes. However, Patient 4 with marked morphological changes had only moderate elevation of resistance, and Patient 21 with mild changes had a high resistance.

In this group six of eight of the patients with a high degree of organic pulmonary arterial change had had one or more severe hemoptyses. On the other hand, patients

such as Nos. 20 and 34 who had had repeated attacks of hemoptysis and No. 12 who had had one severe attack had only mild or moderate vascular disease in the lungs.

An important point to decide in assessing patients in regard to operation is whether or not active rheumatic infection exists. We have observed several patients in whom biopsy specimens of the left auricular appendage indicated active rheumatic infection, as judged by Aschoff bodies, who had no other evidence of activity elsewhere in the heart at the time of post-mortem examination a few hours later. In this particular group there were two such cases (Patients 21 and 22). In addition, there were two patients in whom evidence of regressing active rheumatic myocarditis was present post mortem in the ventricular muscle. These were Patient 20, who also had a positive biopsy specimen from the left auricular appendage at operation six days before death, and Patient 33, who had minimal evidence of an old regressing lesion. Careful review of the records of these four patients failed to reveal any clinical evidence on which a diagnosis of rheumatic activity could be made, and their courses did not differ from those of other patients without activity. In one, No. 20, the sedimentation rate before operation was elevated to 35 mm. per hour, but such elevation has been occasionally observed by us in patients who do not have rheumatic activity.

COMMENT

The findings as described are consistent with those reported by others [11] and with our preliminary conclusions presented in an earlier communication.[1b] The abnormalities in cardiovascular dynamics and many of the organic anatomic changes stem from the mechanical barrier posed by the stenotic mitral valve to the free entry of blood into the left ventricle. The presence, in addition, of a certain degree of mitral insufficiency tends to reinforce the altered cardiac dynamics produced by the stenosis.

The cardiac output is a product of the ventricular discharge per beat and the heart rate. Under normal conditions the control of the ventricular output is dependent on a number of factors, among which are intrinsic factors within the heart muscle itself, the degree of peripheral resistance, and the inflow volume and pressure. In mitral stenosis the chief limiting factor in blood flow becomes the flow through the narrowed mitral ring, and this is dependent on the size, shape, and depth of this orifice, the diastolic head of pressure of blood in the left auricle, and the duration of diastole. This pressure head may to some extent be augmented by auricular systole in patients with normal sinus rhythm, but is mainly the result of the accumulation of blood in the left auricle and pulmonary circulation, which has been pumped in by the right ventricle to a greater extent than it can drain off through the mitral valve. Pulmonary congestion builds up until the increased left auricular pressure is able to equalize mitral-orifice outflow with the inflow from the right ventricle, or until the right ventricular output is lessened by the increase in pulmonary vascular

11. (*a*) Gorlin and others.[6] (*b*) Hickman and Cargill.[10b] (*c*) Borden, C. W.; Ebert, R. V.; Wilson, R. H., and Wells, H. S.: Pulmonary Hypertension in Heart Disease, New England J. Med. **242**:530 (April 6) 1950. (*d*) Bayliss, R. S.; Etheredge, M. J., and Hyman, A. L.: Pulmonary Hypertension in Mitral Stenosis, Lancet **2**:889 (Dec. 30) 1950. (*e*) Gorlin, R.; Sawyer, C. G.; Haynes, F. W.; Goodall, W. T., and Dexter, L.: Effects of Exercise on Circulatory Dynamics in Mitral Stenosis, Am. Heart J. **41**:192 (Feb.) 1951. (*f*) Draper, A.; Heinbecker, R.; Daley, R.; Carroll, D.; Mudd, G.; Wells, R.; Fallolt, W.; Andrus, E. C., and Bing, R. J.: Physiologic Studies in Mitral Valvular Disease, Circulation **3**:531 (April) 1951.

resistance, which may, in turn, lead ultimately to failure of the myocardium of the right ventricle. At the same time, a small output from the left side of the heart inevitably leads to an equivalent small venous return to the right auricle, and this, too, helps to set the level of the right ventricular discharge at a reduced amount which will be in balance with the mitral-valve flow. Thus, at rest, we have the circulatory dynamic conditions which are shown to exist in these studies: first, a consistent elevation in the pressure in the pulmonary circulation, and second, a tendency for the cardiac output to be somewhat reduced.

When the oxygen consumption of the body is increased by exercise or excitement or by any other mechanism, the oxygen need of the tissues in the periphery obviously becomes greater. In patients with normal circulation these needs are met by an increase in blood flow. Normally this rise in output is accomplished by a step-up in the average stroke volume output of the ventricle and by a more rapid heart rate. In mitral stenosis, however, the output from the right ventricle tends to increase to a greater extent than that from the left because of the mechanical barrier of the stenotic mitral valve; or, to put it another way, the amount of blood pumped into the lungs by the right ventricle tends to be greater than the runoff of blood from the lungs through the mitral valve—hence, the tendency toward the increase in pulmonary congestion. An increase in left intra-auricular pressure would tend to augment the flow through the mitral valve into the left ventricle to some extent, but this usually is inadequate.

An attempt is made by the circulation of these patients to redress the balance by a disproportionate increase in heart rate, but this proves to be self-defeating. We have not yet elucidated the mechanism which is responsible for this unusual increase in heart rate. It may arise from reflexes from the periphery or from the vasomotor center itself as a result of the failure of the blood flow to keep up to the oxygen demand, or it may in part be caused by stimuli arising in the stretched auricular musculature itself. In any event this tachycardia has evil consequences, for, if excessive, it tends to reduce the output per beat so greatly that the minute blood flow itself may fail to increase in spite of the rapid heart rate. The explanation for this has been discussed previously [1b] and is as follows: Because of the chronic distention in the left auricle, the flow of blood through the mitral valve is at a maximum throughout diastole in the presence of auricular fibrillation, and, even when sinus rhythm obtains and there is effective auricular contraction, that part of diastole preceding auricular systole is still of the greatest importance since it represents, in time, all but a fraction of diastole. With an increase in heart rate, diastolic time is shortened and that portion of any given time period, such as a minute, devoted to diastole, is much decreased. Hence, the output per beat, and often per minute, is diminished in spite of the increase in left auricular pressure. If mitral insufficiency is present, as well as stenosis, and regurgitation of blood occurs, this will enhance the tendency for tachycardia to reduce the volume output. The regurgitant jets occur with each systole of the heart. The more heart beats per minute, the greater will be the amount of regurgitation, with a consequent diminution in peripheral blood flow and increase in pulmonary congestion.

The embarrassment to the flow of blood out of the lungs, with its inevitable tendency toward congestion in the lung vessels, results in changes in pulmonary vascular pressure which are fully as important as changes in blood flow. The elevated pressure in the left auricle is transmitted back through the lung vessels to the

pulmonary artery. Since the pulmonary vascular reservoir is already distended with blood and since in many cases secondary functional or organic alterations have occurred in the pulmonary blood vessels or in the interalveolar tissues which prevent further dilatation of this pulmonary vascular reservoir, any condition leading to an increase in right ventricular output or to an impairment of flow through the mitral valve, such as occurs with exercise, excitement, or any other state leading to tachycardia, will cause a further increase in the pulmonary artery pressure which frequently is very marked.

In addition to the dynamic changes in the circulation occurring directly as the result of the mitral stenosis, secondary functional or organic alterations in the circulation may take place and may be of importance in determining the clinical picture and course of the disease. There may be an increase in pulmonary artery pressure out of proportion to the back-pressure effect stemming from the raised left intra-auricular pressure. This additional increment in the pulmonary circuit is produced by reflex constriction or by organic narrowing of the small pulmonary vessels, or both. The chronic strain on the right ventricle as the result of the sustained pulmonary hypertension may cause hypertrophy of this ventricle. The majority of our patients showed electrocardiographic evidence that this had occurred. Ultimately this ventricle may dilate and fail, with or without functional tricuspid insufficiency. There will then occur elevated venous pressure, hepatic congestion and enlargement, and an increased tendency for fluid retention, both generally and in serous cavities, especially the peritoneal cavity. Almost all these patients have low cardiac outputs.

Fluid retention and edema may also be present when there is no evidence of right ventricular failure or of an abnormal venous pressure. Usually this type of fluid retention is not marked in degree, but it may be of great importance since it may be clinically manifested mainly in the increase in pulmonary congestion and an aggravation of dyspnea and other symptoms referable to the lung. Five of the patients studied by us had such a tendency to accumulate edema fluid with a normal right auricular or venous pressure response. In only one of them was the liver palpable, and it was felt but 1 cm. below the costal margin. At the time of the study the edema in these patients was minimal, but all required rigid dieting, salt restriction, and diuretic measures, including repeated injections of mercurial preparations, to control the accumulation of fluid. It is, of course, not possible to say that the venous pressure response may not have been abnormal at other times, though this was never observed and in at least two of the patients repeated tests of resting venous pressure were carried out over a period of weeks at times that they did have edema. Although in three the cardiac output was very low, in the other two it was well maintained, with the result that no conclusions can be drawn from this measurement. The cause and the exact nature of this fluid retention have not been further investigated by us in this study.

The physiologic factors measured which showed the closest correlation with the degree of dyspnea experienced by the patient were the level of the pulmonary artery pressure and pulmonary resistance, particularly during exercise. Such correlations have been suggested before.[11e-e] This is in conformity with the hypothesis that the dyspnea of left ventricular failure and of mitral stenosis is due to changes within the pulmonary circulation, resulting in alteration in pulmonary function. Although there

were exceptions to the general tendency for a correlation between the height of the pulmonary artery pressure or pulmonary resistance and the degree of dyspnea, it is perhaps surprising that the correlation held as well as it did in view of the obviously subjective nature of the symptom, dyspnea, and the secondary or associated pulmonary changes which may be present. Thus, organic changes in the alveoli and pulmonary vessels, such as interalveolar edema and fibrosis, may affect pulmonary function and the degree of dyspnea without a direct relation to the pulmonary artery pressure. However, as discussed earlier, high-grade organic changes in the pulmonary circulation are not necessarily accompanied either with severe dyspnea or with unusually elevated pulmonary artery pressure. The condition of Patient 4 is a case in point. It is obvious from our studies that the vital capacity is not a guide of this disturbance in pulmonary function, since there was little correlation between the degree of dyspnea on effort and the vital capacity. A more intensive study of the pulmonary function in mitral stenosis is now being carried on in this laboratory.

It was thought that possibly the arterial oxygen saturation at rest or during exercise might give some clue to pulmonary dysfunction. In most of the patients, regardless of the severity of dyspnea, the resting saturation was only slightly below the normal of 95% and improved with exercise. In eight patients it fell below 90%, and in all but one of these chronic dyspnea was marked. This one exception was a woman with an associated asthmatic bronchitis who exhibited many fine musical rhonchi throughout the lungs. In one patient with a huge left auricle and evidence of atelectasis of the lower lobe of the right lung the arterial saturation was 88%. Three months later, after mitral valvuloplasty, which gave moderate relief of symptoms, the saturation was 97%. However, in many other patients with similar clinical pictures the arterial oxygen saturation was essentially normal. Gorlin and his associates [6] have pointed out the unreliability of arterial oxygen unsaturation as a measure of pulmonary dysfunction.

It is of interest that several of the patients studied had bronchial expiratory obstruction, as shown by asthmatic wheezes and rhonchi, especially during exacerbations of their pulmonary symptoms. Cough was another troublesome symptom. Although cough may have been due to the pressure of an enlarged left auricle on the recurrent laryngeal nerve or distended left pulmonary artery, much more important in the cases studied were irritative stimuli arising more distally in the bronchi or lungs. The beneficial effect of treatment with mercurial diuretic drugs on the cough, wheezing, and dyspnea was striking in many of the patients. Such benefit often occurred even though the diuresis produced a loss in weight of only 2 or 3 lb. (0.9 to 1.4 kg.).

Eleven of the patients had had severe attacks of acute pulmonary edema. In two there had been single attacks associated with pregnancies, five years and three months, respectively, prior to studies. The others had had multiple attacks requiring numerous hospital admissions, although none was studied for several days or more after such an attack. The degree of chronic dyspnea which these patients endured varied from mild to severe. The pulmonary edema was often associated with infections of the upper respiratory tract, which may have initiated it either by a direct effect on the alveolar membrane or by a more general effect in enhancing fluid retention. Other physiologic states associated with a tendency to retain fluid were also of critical importance as factors in the precipitation of pulmonary edema or

aggravation of dyspnea. Thus, the premenstrual period was associated with increase of dyspnea in two patients, and in two others pulmonary edema occurred for the first time during early pregnancy.

Pulmonary edema or marked dyspnea was also associated with any conditions leading to marked tachycardia, such as exercise or excitement. The physiologic explanation for this has already been discussed. It was not possible, however, to show that these patients responded to mild exercise with a greater increase in heart rate than many other patients not subject to pulmonary edema.

Only two of this group of patients had clear-cut right-sided failure at the time of the studies or during the attacks of pulmonary edema, although careful studies during such attacks were not made. One of these (Patient 35) had had violent attacks of pulmonary edema for two years with no peripheral congestion. In the third year he had right-sided failure, with probable tricuspid regurgitation; his rhythm shifted from one of sinus origin to auricular fibrillation, and then his cardiac output fell. He continued to have attacks of pulmonary edema, but these were much less severe than previously. It is of interest that his pulmonary "capillary" pressures, and his total pulmonary and pulmonary arteriolar resistances, were only moderately elevated at the time that he was subject to violent attacks of pulmonary edema and his right ventricle was competent, but that at the later stage, when he was in right ventricular failure, these measurements increased considerably. In 1948 an interatrial septal opening was made, with temporary improvement only. In December, 1949, a finger-fracture mitral valvuloplasty was performed, and since then he has had no further attacks of pulmonary edema, his chronic dyspnea is much less, and the right-sided failure and tricuspid regurgitation are much reduced. He has been studied by cardiac catheterization five times. The other patient had a venous pressure of 250 mm. of water and mild edema of a few weeks' duration only, although his attacks of pulmonary edema had extended over many years.

One other patient had an abnormal increase in right auricular pressure, from a resting level of 0.5 mm. of mercury to 10.0 mm. on exercise but had never had peripheral edema. A fourth patient had moderate peripheral edema but normal right auricular pressure.

It is of great interest that 10 of these 11 patients were in normal sinus rhythm at the time that they were experiencing severe attacks of pulmonary edema. The patient who was observed only in auricular fibrillation had a low cardiac output and the highest resting pulmonary artery pressure of any of the patients in this series. He also showed the most extreme grade of pulmonary fibrosis of any of this group that were examined post mortem.

There were two other patients who ultimately exhibited auricular fibrillation. One, as mentioned above, who had fibrillation and right-sided failure, had a lessening of the number and severity of the acute attacks. Another was studied, after numerous attacks, in August, 1949, at which time she was in sinus rhythm. She was next seen in July, 1950, one month after her only further attack of pulmonary edema. On this occasion she was in auricular fibrillation; it is not known when it developed.

Another striking observation among this group of 10 patients in sinus rhythm who suffered from severe attacks of pulmonary edema was their well-maintained cardiac outputs and the frequent ability to increase them on exercise. In only one patient was the cardiac index after exercise less than 3.0 l., and in this man it

rose from 2.7 l. at rest to 2.8 l. after exercise. In one other, it was 1.7 l. at rest but exercise was not carried out. These outputs averaged much higher than those of other patients with a comparable degree of chronic dyspnea, whether or not they were in auricular fibrillation. The pulmonary artery pressures were elevated both at rest and during exercise but were of the same order as the pressures determined in other patients.

The fact that patients with mitral stenosis who are subject to acute pulmonary edema may have sinus rhythm has already been observed by Bramwell and Jones,[12] and they suggested that acute failure of the left auricle may be responsible for the initiation of the attacks. Our findings are consistent with such a theory so far as the observations on heart rhythm go in most of our cases. The relatively good cardiac output observed by us in such patients between attacks implies that a mechanism exists for successfully maintaining a good output through the narrowed mitral orifice, and this may be a forceful left auricular contraction. If such a mechanism suddenly fails, then there is an acute disturbance of the balance between pulmonary inflow and outflow.

The explanation attributing an important role to the left auricle is by no means established. It is quite possible that whether a given patient has attacks of acute pulmonary edema or not is dependent on the degree of myocardial damage and insufficiency in relation to the degree of obstruction of the mitral stenosis. If the right ventricular myocardial function is normal, then blood flow may be well maintained, even in the presence of severe mitral stenosis and high pulmonary vascular pressures, and the breaking point then appears in the pulmonary circuit with the development of acute pulmonary edema. If, however, the right ventricle is inadequate, then the breaking point will be right ventricular failure, either acute or chronic.

The well-maintained cardiac output in most patients is as consistent with this explanation as with the first one, and the presence of normal rhythm may be merely an indication that myocardial degenerative processes have not proceeded as far in this group as in patients of the other type. The failure to find an exact correlation with the level of the pulmonary arterial pressure is not surprising. Anyone who has followed the pressures in these patients has observed the rapid and wide fluctuations in pressure which occur from the apparently minor dyspnea of exercise, excitement, and other stimuli. These patients were not studied during their attacks of pulmonary edema, and therefore their pulmonary arterial pressures at such times are not known.

A number of other factors may be operative in determining whether a patient has frank pulmonary edema or merely an increase in dyspnea. Of primary importance is the level of pressure in the pulmonary capillaries. On theoretical grounds, pulmonary edema should result when this level exceeds that of the colloid osmotic pressure of the blood. It has been suggested [13] that a tendency to rapid increase in pulmonary arteriolar resistance, possibly due to the pulmonary arteriolar constriction which occurs when the pulmonary capillary pressure approaches the colloid osmotic pressure of the blood, protects some patients from a high capillary pressure and edema because the increase in resistance blocks the free ingress of blood into

12. Bramwell, C., and Jones, A. M.: Acute Left Auricular Failure, Brit. Heart J. **6**:129 (July) 1944.

13. Gorlin and others.[6] Gorlin and others.[11e]

the pulmonary capillaries and left auricle. Other factors are undoubtedly of importance. The physiologic and structural condition of the alveolar membrane and the interalveolar tissue may play a role in allowing or retarding the passage of fluid across alveolar walls. Factors affecting the permeability of the alveolar membrane must also be considered. Such change in permeability may be brought about by reflex or by hormonal effects.

As stated earlier, it is impossible to predict the extent of organic change in the pulmonary blood vessels or lung tissue from the clinical course or physiologic measurements. All the patients subject to acute pulmonary edema who were examined post mortem had well-marked organic changes, but this was also true of the majority of the autopsied patients without a history of acute pulmonary edema. Whether this correlation would hold in a larger series, we cannot say, nor is there as yet any evidence available concerning the reversibility of some of these pathologic alterations if the dynamics of the pulmonary circulation can be restored to normal by operation on the mitral valve.

The finding of unsuspected active rheumatic myocarditis in some patients in this group demonstrates again what has frequently been pointed out, that the rheumatic state may be present in a cryptic fashion in adult life. To what extent such low-grade activity influences the course of the disease in patients who demonstrably suffer chiefly from the effects of high-grade mitral valve obstruction is also unknown.

SUMMARY AND CONCLUSIONS

A study is reported of 38 patients with mitral stenosis of varying degrees. Physiologic measurements, particularly of the cardiac output and pulmonary vascular pressure, were correlated with the clinical state of the patients. Although the cardiac output tended to decrease with increasing degrees of clinical disability, this did not hold as a strict correlation. A better correlation was found between the degree of disability and the increasing pulmonary arterial pressure and pulmonary resistance, but even here exceptions were present, and there was considerable variation in the absolute levels of the pressures. An explanation of the physiologic mechanisms which produce clinical symptoms and signs is offered. Any condition producing tachycardia tended to impair the circulatory function, frequently by a decreased stroke volume output. Many of the patients showed the typical physiologic findings of right-sided heart failure, namely, increased right auricular and peripheral venous pressure with further increase during exercise. Most of such patients also had signs of chronic congestion. Peripheral edema, however, occurred in some patients without evidence of right-sided heart failure.

Twelve of the patients in whom these studies were made died and were examined post mortem. It was found that the degree of mitral stenosis and the degree of pulmonary vascular change could not be closely or accurately correlated either with the degree of disability of the patient or with decreases in cardiac output or increases in pulmonary pressure, although, of course, there was a general tendency for such correlations to exist. Active rheumatic myocarditis was found at post-mortem examination to be present in several patients in whom it was not clinically diagnosed.

A group of 11 patients subject to attacks of acute pulmonary edema is particularly analyzed. Ten of these patients were in normal sinus rhythm at the time of their severest attacks, and in nearly all the cardiac output was well maintained. The possible mechanisms for the pulmonary edema are discussed.

STUDIES IN MITRAL STENOSIS

II. A Correlation of Post-Mortem Findings with the Clinical Course of the Disease in One Hundred One Cases

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WITH THE development of surgical technics for the relief of mitral stenosis which promise to be both effective and not unreasonably hazardous, it has become important to reassess this syndrome in regard to various pathologic factors that may affect the clinical course of the disease and have a bearing on the selection of patients for operation. Among such factors, the interrelations between the degree of stenosis, the presence of organic pulmonary vascular lesions, the occurrence of emboli, and rheumatic activity, and the manner in which cardiovascular breakdown and death occur are important. This study was undertaken with these points in view.

MATERIALS AND METHODS

Cases studied were from the post-mortem files of the Mallory Institute of Pathology, of the Boston City Hospital, between January, 1945, and June, 1949, and include the autopsy and clinical records of all patients whose conditions was diagnosed at post-mortem examination during this period as rheumatic heart disease with mitral valve involvement. Of this total number, a few were omitted because of one of the following reasons: 1. Restricted autopsy precluded sufficient post-mortem data. 2. In the judgment of the pathologist, the mitral valve was dilated and in no degree stenosed. 3. The clinical record was insufficiently complete to give an adequate amount of reliable data.

Analysis of Records.—All the clinical data on each patient were reviewed. In many cases clinical data comprised only those at a terminal admission, but in numerous instances there had been frequent hospital entries. All the pertinent factors that could be obtained from each of the hospital admissions were reviewed. Data obtained on physical examination were usually taken from the record of the final admission. Patients were classed as having auricular fibrillation if it was present during their final hospital stay. In the majority of cases, the rhythm was confirmed by electrocardiograms, and these were reviewed by one of us.

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The post-mortem protocols were similarly reviewed. As was indicated before, all the patients showed the morphological characteristics of rheumatic heart disease with mitral valvular stenosis.

The degree of stenosis was graded from 1 to 3: A valve with Grade 1 stenosis was described as "admitting two fingers" or had an analogous description; a valve with Grade 2, or moderate, stenosis usually was described as "admitting one or one and a half fingers" or as "admitting the tips of two fingers"; those with Grade 3, or marked, stenosis included valves which were usually described as "admitting only the tip of one finger," or "will not admit one finger," as showing "typical marked fish-mouth stenosis," or "markedly or extremely stenosed." Originally this third group of extremely stenotic valves was subdivided into three further groups, but it proved impractical to make such fine distinctions. Correlations that were made on the basis of such subgroups in general bore out the findings for the group as a whole.

Note was made of the gross description of the lungs, particularly in regard to the presence or absence of fresh or old infarctions and of congestion. The microscopic sections of these lungs were all reviewed in detail by one of us (D. J. G.) and were classified from Grade 1 to 3 on the basis of the organic changes noted as reflecting the congestion produced by the mitral valvular stenosis. The findings which led to this classification are set forth in the article by Parker and Weiss.[1] Further consideration of such changes will be given later in this article in the section devoted to our observations in this regard.

In similar fashion all the sections of the heart were reviewed for the possible presence of acute rheumatic activity in the myocardium.

Criteria for the cause of death were based on both clinical and pathologic evidence, and when any conflict in such evidence occurred the final conclusion was reached after a careful evaluation of the entire record.

RESULTS

Age and Sex.—Forty-eight of the 101 patients were men and 53 were women. The sex distribution of the 50 patients with involvement confined to the mitral valve was equal. This sex distribution is, in general, in accord with that in other studies based on post-mortem material, which have usually indicated that the tendency for mitral valve involvement is slightly more frequent in the female but that aortic valve lesions occur in the male in greater numbers. Sex distribution with regard to autopsies as a whole at the Boston City Hospital during the period of study was 62% males and 38% females, and if adjustment is made for this factor the preponderance of mitral stenosis would be in the female.

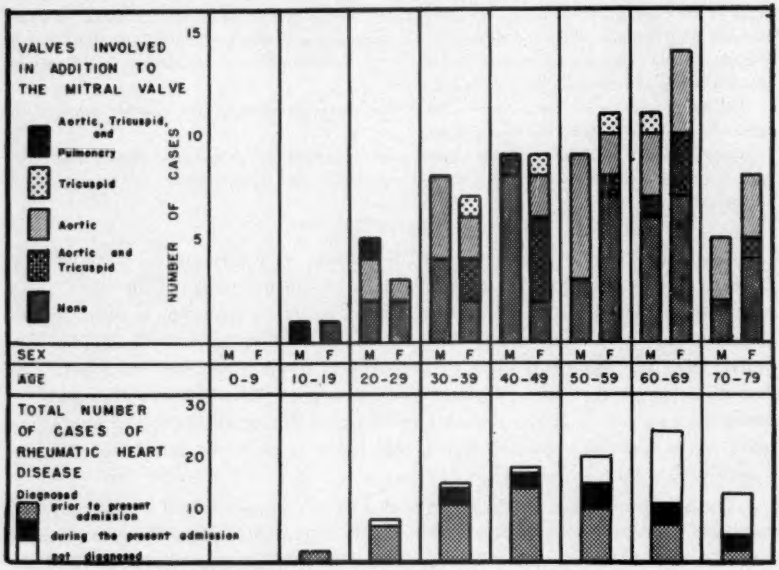
The age distribution, of the group both as a whole and divided according to the valves involved, is shown in the Chart. It is of interest that 58 of the patients were over the age of 50; 38, over 60, and 13, over 70. The average age at death of the women tended to be slightly higher than that of the men, the average age at death being 53 yr. for women and 50 yr. for men. It is to be noted that the age distribution at death of patients with involvement confined to the mitral valve was not dissimilar from that of patients who had aortic involvement also. Our findings in respect to the age of death agree in general with those of Friedberg and Tartakower [2] and Lichtman and Master [3] but are somewhat in variance with those in many of the

1. Parker, F., Jr., and Weiss, S.: The Nature and Significance of the Structural Changes in the Lungs in Mitral Stenosis, Am. J. Path. **12**:573 (Sept.) 1936.

2. Friedberg, C. K., and Tartakower, J.: Über den günstigen Verlauf der endokarditischen Herzklappenfehler, Ztschr. klin. Med. **116**:759, 1931.

3. Lichtman, P., and Master, A. M.: The Incidence of Valvular Heart Disease in People over 50 and Penicillin Prophylaxis of Bacterial Endocarditis, New York J. Med. **49**:1693 (July 15) 1949.

reported series,[4] in which the average age was lower. Few patients under 20 are included in our series, partly at least because comparatively few post-mortem examinations are carried out on children in this hospital. To some extent, too, this variation is due to the manner of case selection in the different studies. Many of the other studies have been based upon groups of patients with known mitral stenosis or rheumatic heart disease studied in cardiac clinics or in private practice. Many persons who have a definite degree of mitral stenosis live out their life span without the presence of rheumatic heart disease being suspected. This is shown in the Chart, in which it is to be seen that in a considerable percentage of patients, particularly of those over 50 yr. of age, the rheumatic heart disease was never diagnosed during life or was diagnosed only during the terminal illness. When patients with no or terminal diagnoses are excluded from the series, it can be seen that the average age



Distribution of cases in relation to age, sex, and valves involved.

of our patients is much lower and is comparable with that in many of the other series reported. Table 1 shows that, although there is some tendency of the younger patients to have a higher proportion of severe, or Grade 3, mitral stenosis, nevertheless, severe mitral stenosis was common in the older age group, since 21 of the

4. (*a*) Davis, D., and Weiss, S.: Rheumatic Heart Disease: II. Incidence and Distribution of Age at Death, Am. Heart J. **8**:182 (Dec.) 1932. (*b*) Jones, E. W.: Etiology and Symptoms of Mitral Stenosis: Review of 300 Cases, Brit. M. J. **1**:307 (Feb. 25) 1933. (*c*) Laws, C. L., and Levine, S. A.: Clinical Notes on Rheumatic Heart Disease with Special Reference to the Cause of Death, Am. J. M. Sc. **186**:833 (Dec.) 1933. (*d*) Stone, C. S., and Feil, H. S.: Mitral Stenosis: Clinical and Pathological Study of 100 Cases, Am. Heart J. **9**:53 (Oct.) 1933. (*e*) DeGraff, A. C.; Lingg, C., and Cohn, A.: The Course of Rheumatic Heart Disease in Adults, ibid. **10**:459 (April) 1935. (*f*) Cohn, A. E., and Lingg, C.: The Natural History of Rheumatic Cardiac Disease: I. Onset and Duration of Disease, J. A. M. A. **121**:1 (Jan. 2) 1943.

58 patients over 50, and 16 of the 38 over 60, had mitral stenosis which was Grade 3 in severity. An analysis of the cases in which there were associated lesions of the aortic or other valves revealed that such lesions had apparently no bearing on the incidence of severe mitral stenosis in respect to the age of the patient at death.

It is of interest that of 47 patients with severe mitral stenosis 15 had either no or slight impairment of cardiac reserve up to the time of their death or until the terminal part of their illness. In three the impairment of functional reserve was Class 2 (American Heart Association Classification) and in eight it was Class 3, whereas in the final 21 patients the functional reserve was so impaired that they were completely incapacitated by heart failure. It is, thus, evident that mitral stenosis of a severe grade may be tolerated by patients for a protracted period with either little or no impairment of cardiac reserve manifested by dyspnea on exertion.

This is also apparent in an analysis of the patients in whom rheumatic heart disease was not diagnosed during life. There were 31 in this group, and six of them had marked mitral stenosis. All six of these, and 21 of the total of 31, were able to carry on with either no cardiac symptoms or with mild dyspnea only, prior to their terminal illness.

TABLE 1.—*Relation of Age to Severity of Mitral Stenosis*

Age, Yr.	Severity of Mitral Stenosis			Total	% with Grade 3 Stenosis
	Grade 1	Grade 2	Grade 3		
10-19	1	0	1	2	50
20-29	2	0	6	8	75
30-39	2	7	6	15	40
40-49	1	4	13	18	75
50-59	4	11	5	20	25
60-69	6	6	13	25	52
70+	5	5	3	13	25
Total	21	33	47	101	46

It was impossible to determine the duration of the mitral stenosis in these patients because the clinical records were inadequate in dating the onset of the disease. In addition, of course, there was no way of determining how long the mitral stenosis was of the severe grade that was found at post-mortem examination. Obviously, however, the mitral stenosis must have been of this degree for months at least before death, and it was tolerated by many of the patients as shown above with surprisingly little difficulty. In a study of the development of mitral stenosis in young persons, Walsh, Bland, and Jones [5] pointed out that the fully developed clinical signs of this condition do not usually become manifest for five to 15 years after the initial rheumatic infection, which in most instances is of mild nature. What the conditions are that determine the progressive change in the size of the mitral valve until true stenosis ultimately results is unknown, and, in particular, it is not known to what extent active rheumatic recurrences, as contrasted with the contraction of scarred valve margins, are involved. If the process takes so many years to develop after the initial infection before it is clinically recognizable, it can be assumed that in many instances the process of narrowing will continue for many years thereafter. The present study throws no light on this problem, of course, and our records were not adequate for a proper analysis of a past history of rheu-

5. Walsh, B. J.; Bland, E. F., and Jones, T. D.: Pure Mitral Stenosis in Young Persons, Arch. Int. Med. **65**:321 (Feb.) 1940.

matic fever. However, in only one patient in our series were active vegetations of rheumatic origin seen on the mitral valve at post-mortem examination, and this was the patient whose death was caused by rheumatic myocarditis.

The incidence of auricular fibrillation was somewhat but not greatly higher with increasing severity of the mitral stenosis; it was present in 52% of the patients with Grade 1 mitral stenosis, in 59% with Grade 2, and in 62% with Grade 3. Similar results were reported by de la Chapelle, Graef, and Rottino.[6]

The clinical records were not adequate to give a good estimate of the length of time that the patients had complained of dyspnea or any cardiac disability, but so far as the records could be analyzed there was no correlation between the severity of the stenosis of the mitral valve and the length of time that symptoms of cardiac disability had existed.

CAUSE OF DEATH

Heart failure of one type or another accounted for deaths of 44 of the 101 patients (Table 2). Of these, eight died as the direct result of subacute bacterial

TABLE 2.—*Cause of Death*

	No. of Patients	Average Age	No. with Grade 3 Stenosis
Heart failure	**44**	**50**	**21**
Congestive failure	13	..	8
Acute pulmonary edema	11	..	5
Sudden death	4	..	3
Subacute bacterial endocarditis	8	..	2
Acute bacterial endocarditis	3	..	..
Active rheumatic fever	1	..	..
Postdelivery	2	..	1
Postoperative: Valvular surgery	2	..	2
Deaths due to peripheral disorders	**33**	**56**	**18**
Cerebrovascular accidents	26	..	15
Arterial occlusions in other areas	4	..	1
Multiple renal infarctions	3	..	2
Pulmonary emboli	11	45	4
Pneumonia	6	..	2
Deaths due to noncirculatory disorders	7	..	2
Total	101	52	47

endocarditis; in three acute bacterial endocarditis was present; two died in heart failure following delivery, and two died during operation on the mitral valve. In only one patient was active rheumatic fever considered to be the direct cause of death. In this case it was diagnosed during life. The problem of rheumatic activity will be discussed later.

In 33 patients acute events in the peripheral circulation caused death. Twenty-six of these died of cerebrovascular accidents; in two occlusion of the abdominal aorta and in two occlusion of a mesenteric vessel occurred. Three died in renal failure and at post-mortem study showed multiple infarctions of the kidneys. In this group of patients emboli were the responsible factors in many deaths, and undoubtedly emboli that could not be demonstrated contributed to the deaths of other patients. The problem of embolization will be discussed subsequently. Pul-

6. de la Chapelle, C. E.; Graef, I., and Rottino, A.: Studies in Rheumatic Heart Disease: Analysis of 119 Hearts with Special Reference to the Relationship of Auricular Fibrillation to Mitral Valvular Deformity and Certain Rheumatic Tissue Changes, Am. Heart J. **10**:62 (Oct.) 1934.

monary infarction accounted for 11 deaths, and in 20 more patients fresh pulmonary infarctions, which may have contributed significantly to the final outcome, were seen at post-mortem examination. Six died of pneumonia, and it is not evident what the role of a failing heart may have been in these patients. Aside from the patients with pneumonia, there were only seven who died of causes clearly unrelated to the heart or circulation.

The causes of deaths in patients with severe, or Grade 3, mitral stenosis is also shown in Table 2. It is seen that in these patients the distribution of causes of death does not differ greatly from that for the group as a whole. Although there was some variation in the percentage of patients in each group that had auricular fibrillation, the series was too small for such variation to be of any significance.

Our findings regarding the causes of death do not differ significantly from those reported by others, although there has been considerable variation in the percentages of causes of death as reported for different series. Stone and Feil,[4d] however, found that 81% of their patients died in congestive heart failure and only 5% had bacterial endocarditis. In contrast, Laws and Levine [4c] reported that only 33% of their patients died of congestive failure, 23% of acute rheumatic fever, and 29% of subacute bacterial endocarditis, with 11.5% dying of emboli. Undoubtedly, the manner of selecting patients, the age of the patients who were observed, and the period in which the study was made, particularly in regard to the treatability of bacterial endocarditis, explain the variations in the findings in the different studies. Nearly 20 years ago a study of the incidence of rheumatic heart disease, as revealed by post-mortem observations, was made by Weiss and Davis [7] at this hospital. At that time 41 patients had definite or suspected subacute bacterial endocarditis and 23 more had malignant endocarditis of a total of 474 patients with rheumatic heart disease, 269 of whom died as the result of heart disease. The patients with subacute bacterial endocarditis represented, therefore, 8.6% of the entire group, or 15.3% of those who died of heart disease. Although the selection of patients and the analysis of data in these authors' series was not strictly comparable with ours, it is of interest that there is a substantial incidence of deaths resulting from subacute bacterial endocarditis (8%) even in these days of chemotherapy.

An analysis of our eight cases of subacute bacterial endocarditis is as follows:

For four patients the clinical diagnosis of subacute bacterial endocarditis was made at their final hospital entry before death. One of these patients died of heart failure and three of their infection in spite of penicillin therapy. Blood cultures in two showed alpha Streptococcus and in the other two were sterile (post-mortem blood culture in one of these revealed Salmonella choleraesuis). A fifth patient had been treated for subacute bacterial endocarditis two years prior to death, but the disease was not correctly diagnosed at his terminal admission, which was for heart failure. Blood cultures for this patient were not taken. Another patient had been treated for clinically proved subacute bacterial endocarditis 18 months prior to his sudden death, which occurred after progressive renal and mild cardiac failure, during which he repeatedly had showers of petechiae. Post-mortem examination revealed healed subacute bacterial endocarditis and healed focal embolic glomerulonephritis.

7. Davis, D., and Weiss, S.: Rheumatic Heart Disease: I. Incidence and Role in the Causation of Death: Study of 5,215 Consecutive Necropsies, Am. Heart J. **7**:146 (Dec.) 1931.

In the final two cases the diagnosis of endocarditis was not suspected clinically. In one the diagnosis was meningitis, and at autopsy the meningitis and endocarditis were discovered to be due to a Salmonella organism. The second patient had a clinical and blood picture consistent with pernicious anemia and died with a subdural hemorrhage.

In only one of the three patients with acute bacterial endocarditis was the diagnosis made clinically. Death of these three patients resulted from multiple embolism, from pneumonia, and from shock, respectively.

EMBOLIC PHENOMENA AND INTRACARDIAC AND VENOUS THROMBOSIS

An analysis was made of the occurrence of emboli and infarctions, both to the periphery and to the lungs, in relation to the occurrence of auricular and other thrombi and to the cardiac rhythm. These included not only cases of fresh infarctions and emboli clearly demonstrated at post-mortem study but also cases in which there was pathologic evidence of old infarctions, together with unequivocal

TABLE 3.—*Peripheral Infarctions*

	Total	No. with Auricular Fibrillation	Sinus Rhythm
Recent infarctions without bacterial endocarditis	27	27	6
With thrombi in left atrium	3	3	0
With thrombi in left auricular appendage	5	5	0
With thrombi in both atrium and appendage	1	1	0
No auricular thrombi	18	12	6
Recent infarctions with bacterial endocarditis	6	2	4
Old infarctions without bacterial endocarditis	12	11	1
With thrombi in left atrium	2	2	0
With thrombi in left auricular appendage	2	2	0
No auricular thrombi	8	7	1
Old infarctions with bacterial endocarditis	4	3	1
Total	49	37	12
Total patients studied	101	61	40

clinical evidence of embolization prior to death. The most difficult problem concerned the analysis of the etiologic factors in cerebrovascular accidents. These cases are included in the analyses given below and were assumed to be embolic in origin only under one of the following conditions: (1) the demonstration of an embolus at post-mortem examination or (2) the absence of cerebral arteriosclerosis or hemorrhage. It is probable that numerous embolic episodes occurred which are not listed here because of inadequate clinical and pathological evidence.

Peripheral infarctions clearly occurred in 49 of the patients, and in 37 (76%) of these auricular fibrillation was present (Table 3). In 10 patients infarctions were associated with bacterial endocarditis. In two-thirds of the patients with both recent and old infarctions who did not have bacterial endocarditis, no thrombus was present in the left auricle at post-mortem examination. The possibility cannot, of course, be excluded that in a few of these cases the infarction was due not to an embolus but to some other cause, such as a local thrombosis. Moreover, the site of origin of the embolus may have been other than the left auricle; the alternative explanation is that the thrombus which caused the embolus was completely detached from the auricle. In every case in which thrombus was present in the auricle of a patient showing infarctions, auricular fibrillation was present.

When the problem of the interrelationship of peripheral embolization and auricular thrombosis is looked at from the opposite point of view, as shown in Table 4, it is seen that 33 of the patients had thrombi present in either the left atrium or the left auricle appendage, or both, and that all but three of these patients were in auricular fibrillation. In 15, or somewhat less than half of these patients, there was clear evidence that peripheral emboli had occurred, although in two of the patients bacterial endocarditis was also present.

In general, our results agree with those on other studies.[8] This aspect of the problem of peripheral embolization and auricular thrombosis has been analyzed at some length because emboli are so frequently the cause of death or of major disability to these patients, and this approach to the problem becomes of prime importance in analyzing patients who may be candidates for surgical procedures, in relation both to the significance of embolization in the life history of the disease and to the added danger of dislodgment of thrombi during operation on the mitral valve of patients with auricular fibrillation. The problem also bears on the possi-

TABLE 4.—*Incidence of Left Auricular Thrombi*

Site of Thrombi	No.	No. with Auricular Fibrillation	Sinus Rhythm
Left atrium	18	11	2
With peripheral infarctions			
Recent	3	3	0
Old *	4	3	1
Left auricular appendage	15	15	0
With peripheral infarctions			
Recent	5	5	0
Old	2	2	0
Left atrium and auricular appendage	5	4	1
With peripheral infarctions	1	1	0
Total	33	30	3
Total patients studied	101	61	40

* Two patients also had bacterial endocarditis.

bility of surgical resection or ligation of the left auricular appendage designed to diminish the likelihood of further embolization, as suggested by Madden [9] and Baronofsky and Skinner.[10] Since thrombi were present in and confined to the left auricular appendage in such a small proportion of our patients who had evidence of peripheral emboli, it does not appear that these surgical procedures would be helpful in preventing further embolization.

8. Harvey, E. A., and Levine, S. A.: A Study of Uninfected Mural Thrombi of the Heart, Am. J. M. Sc. **180**:365 (Sept.) 1930. Weiss, S., and Davis, D.: Rheumatic Heart Disease: III. Embolic Manifestations, Am. Heart J. **9**:45 (Oct.) 1933. Graef, I.; Berger, A. R.; Bunim, J. J., and de la Chapelle, C. E.: Auricular Thrombosis in Rheumatic Heart Disease, Arch. Path. **24**:344 (Sept.) 1937. Garvin, C. F.: Mural Thrombi in Heart, Am. Heart J. **21**:713 (June) 1941. Söderström, N.: Myocardial Infarction and Mural Thrombosis in the Atria of the Heart, Acta med. scandinav., Supp. 217, p. 1, 1948. Jordan, R. A.; Scheifley, C. A., and Edwards, J. E.: Mural Thrombosis and Arterial Embolism in Mitral Stenosis: Clinical Pathologic Study of 51 Cases, Circulation **3**:363 (March) 1951.

9. Madden, J. L.: Resection of the Left Auricular Appendix: Prophylaxis for Recurrent Arterial Emboli, J. A. M. A. **140**:769 (July 2) 1949.

10. Baronofsky, I. D., and Skinner, A.: Ligation of Left Auricular Appendage for Recurrent Embolization, Surgery **27**:848 (June) 1950.

Hay and Levine [11] pointed out that age, as well as the presence of auricular fibrillation, is a determining factor in the precipitation of emboli. Our findings, although not statistically significant, are in agreement with this, since 70% of the patients who had had embolism were over 50 (excluding patients with bacterial endocarditis), with 56 yr. the average age for the group. The average age of death for the entire series was 52 yr., and for patients with pulmonary infarctions it was somewhat lower, being 46 yr.

In 31 patients there was clear evidence of recent pulmonary infarction (Table 5), and in 14 of these patients a possible source of pulmonary embolus either in the right auricle or in the peripheral vein, or both, was discovered. Three of the patients in whom no source was found, however, had post-mortem examinations restricted to the chest, and, undoubtedly, in many of the other post-mortem examinations extensive search of the peripheral veins for possible thrombosis was

TABLE 5.—*Incidence of Pulmonary Infarctions and Right Auricular Thrombi*

	No.	No. with Auricular Fibrillation	Sinus Rhythm
Pulmonary infarctions	31	22	9
With right auricular thrombi	7	7	0
With peripheral vein thrombosis	5	2	3
With both right auricular and peripheral vein thrombi	2	2	0
No source found	17	11	6

TABLE 6.—*Incidence of Right Auricular Thrombi*

Site of Thrombi	No.	No. with Auricular Fibrillation	Sinus Rhythm
Right atrium	3	2	1
With pulmonary infarction	3	2	1
Right auricular appendage	7	7	0
With pulmonary infarction	4	4	0
Right atrium and auricular appendage	2	2	0
With pulmonary infarction	2	2	0
Total patients studied	101	61	40

not made. The incidence of thrombosis in the right auricle was considerably less than in the left, since right auricular thrombosis was seen in only 12 patients (Table 6). Six of these 12 patients had involvement of the tricuspid valve, and in 5 of these 6 chronic congestive failure had been present. Of the remaining six patients without right-sided valvular involvement, five had had chronic congestive failure for years. In only one patient was peripheral congestion absent, and this was the one patient of the 12 who was not in auricular fibrillation. In this patient pulmonary congestion and pulmonary infarction were present, and she died of a cerebral embolus.

CONGESTIVE FAILURE

In 71 of the patients congestive heart failure either was present at death or had clearly been present prior to death. All the patients who showed tricuspid involvement had had congestive heart failure. Seventy-two per cent of patients

11. Hay, W. E., and Levine, S. A.: Age and Auricular Fibrillation as Independent Factors in Auricular Mural Thrombus Formation, Am. Heart J. **24**:1 (July) 1942.

with involvement of the mitral valve alone had congestive heart failure, in contrast to 40% of those who had involvement of both the mitral and the aortic valves.

Auricular fibrillation occurred in 47 of the 71 in congestive failure and was noted in only 14 of the 30 patients who did not have congestive heart failure. When the involvement was confined to the mitral valve, auricular fibrillation was more than twice as frequent as sinus rhythm in patients with failure, in contrast to the group with involvement of the mitral and aortic valves in whom sinus rhythm was commoner. All but three of the patients with involvement of the tricuspid valve showed auricular fibrillation.

Embolic phenomena were common in the patients with congestive failure; 23 of the 71 showed recent pulmonary infarctions, and 27, peripheral infarctions, with emboli to both the lung and the periphery occurring in five patients.

RIGHT VENTRICULAR HYPERTROPHY

Some estimate of the degree of right ventricular hypertrophy can be gained from the thickness of the right ventricle as routinely measured at post-mortem examination. This is admittedly not very accurate, but, nevertheless, there was a definite tendency for the degree of right ventricular hypertrophy to be related to marked mitral stenosis, as 11 of the 12 patients with a right ventricle which

TABLE 7.—*Degree of Mitral Stenosis Versus Thickness of Right Ventricle*

Thickness of Right Ventricle, Cm.	Total Patients	Degree of Mitral Stenosis		
		Grade 1	Grade 2	Grade 3
Less than 0.5	61	15	25	21
0.6 to 0.9	27	5	7	15
1.0 and greater	12	0	1	11
Total	100 *	20 *	33	47

* In one patient with Grade 1 stenosis the right ventricle was not measured.

measured 1.0 cm. or more in thickness had marked mitral stenosis (Table 7). There was no correlation between organic changes in the lung and the thickness of the right ventricle.

HEMOPTYSIS

Fifteen patients had had hemoptyses prior to the terminal admission. In 16 patients hemoptysis of some degree had occurred during the final admission or just prior to it. There was no particular relation between the grade of mitral stenosis and the occurrence of hemoptysis during the terminal illness, but hemoptysis occurring prior to the terminal illness was commoner in patients with severe mitral stenosis, since 11 of the 15 instances were in patients with Grade 3 stenosis. Nine had taken place within three years of the time of death, and eight of these nine within two years of death. In six instances hemoptysis had occurred more than five years prior to death. These findings are in conformity with the conclusion expressed by Wolff and Levine[12] that hemoptysis is an ominous symptom in patients with mitral stenosis. There was no correlation between the occurrence of hemoptysis and the presence of organic changes in the lung and pulmonary circulation, since nine of the 15 patients with hemoptysis prior to the terminal illness had either no or minimal changes in the pulmonary circulation.

12. Wolff, L., and Levine, H. B.: Hemoptysis in Rheumatic Heart Disease, Am. Heart J. **21**:163 (Feb.) 1941.

ROLE OF PREGNANCY

Of the 53 female patients, 15 had never been pregnant. In eight the clinical records were not clear on whether or not pregnancies had occurred. Thirty patients had had one or more pregnancies. Six patients had had one pregnancy; eight, 2; four, 3; five, 4; two, 5, and one each, 6 and 7. Two had been pregnant 10 times and one 12 times. The last patient died at the age of 67, with the record of having had no definite evidence of heart failure, after a brief illness following an embolus to a leg. At post-mortem study her mitral valve showed Grade 3 stenosis. One patient who had had 10 pregnancies died at the age of 47. Post-mortem examination showed extreme obstruction of the mitral orifice. She had heart failure during her last two pregnancies. The other patient who had had 10 pregnancies died at the age of 54 and showed Grade 2 mitral stenosis.

Two patients died in congestive heart failure a few hours following delivery. In two others, including one mentioned above as having had 10 pregnancies, heart failure occurred during pregnancy. There is no other record of heart failure occurring during a pregnant period in any of the other patients. The average age at death of the women who had had one or more pregnancies was 58 yr., in contrast to the average age of 53 for the entire group of women. This finding is in

TABLE 8.—*Hemoptysis*

Hemoptysis Prior to Terminal Illness	Total No. of Patients Studied	Degree of Mitral Stenosis		
		Grade 1	Grade 2	Grade 3
	101	21	33	47
Number	15	1	3	11
Per cent	15	5	9	23
Hemoptysis During Terminal Illness				
Number	16	2	6	8
Per cent	16	10	18	17

agreement with other reports.[13] There are, of course, so many factors concerned that a study of this type sheds little light on the effect of pregnancy on the course of the disease in any given patient, with the exception of the two patients in whom a fatal outcome was unquestionably precipitated by the pregnancy or delivery, and the two in whom heart failure developed during pregnancy.

ORGANIC CHANGES IN THE LUNG AND PULMONARY CIRCULATION

As stated earlier, all the microscopic slides of the lung were reviewed by one of us (D. J. G.). Since these were routine sections, usually there were only one or two of each lung, ordinarily taken from the lower lobes and stained only with hematoxylin and eosin. A separate analysis of sections marked specifically "lower lobe," as compared with the series as a whole, revealed no significant change in the observations. The gradation of changes as observed was the same as that described by Parker and Weiss[1] and is as follows: Mild or Grade 1 changes consisted of dilatation of the capillaries to an extent that their width would accommodate five or six red blood cells, apparent increase in the number of capillaries, herniation of the capillaries in the alveolar spaces, and slight thickening of capillary basement membrane. Moderate or Grade 2 changes consisted of a

13. Friedberg and Tartakower.[2] Stone and Feil.[6d] Hamilton, B. E., and Thompson, K. J.: The Heart in Pregnancy and the Child-Bearing Age, Boston, Little, Brown & Company, 1941.

greater degree of thickening of the capillary basement membrane, interstitial edema, and slight thickening and increase in interstitial collagen. Capillary dilatation persisted, but there tended to be less herniation of the capillaries into the alveolar spaces. Grade 3 changes were an increase in interstitial connective tissue and a definite increase in the capillary basement membrane. The capillaries tended to be separated from the alveolar spaces by interstitial collagen, and there was a change in the type of capillary epithelium to the cuboidal form. The capillaries themselves in these thickened walls appeared to be small and in some instances could not be made out. Intimal thickening of the arteries and the hyperplastic arteriolosclerosis and arteriolonecrosis tended to become increasingly marked with greater degrees of change in the alveolar walls. As a rule, both pulmonary vascular and parenchymatous changes were most pronounced in those lungs that showed infarction.

Table 9 shows the relation between the degree of mitral stenosis and the pulmonary changes. All but two of the 14 patients with marked pulmonary changes and all but three of the 22 patients with moderate changes showed either Grade 2 or Grade 3 mitral stenosis. It appears, therefore, that well-marked pulmonary changes are uncommon in the absence of a considerable degree of mitral stenosis.

TABLE 9.—*Degree of Mitral Stenosis Versus Organic Pulmonary Changes*

Degree of Organic Pulmonary Changes	Total Patients	Degree of Mitral Stenosis		
		Grade 1	Grade 2	Grade 3
None	22	3	7	12
Mild	37	10	10	17
Moderate	22	3	9	10
Marked	14	2	6	6
Total	95 *	18 *	32	45 *

* Microscopic sections of the lungs of three patients with Grade 1, one with Grade 2, and two with Grade 3 stenosis were not available for review.

However, mitral stenosis of advanced degree can exist with virtually no organic alteration in the pulmonary circuit, since 12 of the 45 patients with marked mitral stenosis had no demonstrable organic changes in the pulmonary circulation, and in 17 the changes were only mild.

When the ages of the patients at death were analyzed in relation to the degree of pulmonary changes, as shown in Table 10, patients who escaped organic changes in their lungs were found to be among the older group; otherwise there was no good correlation between the age and such changes. The records not only did not permit an accurate analysis of the duration of symptoms but also failed to reveal any definite correlation between the length of time the patients had complained of dyspnea or other evidence of cardiac disability and the degree of organic change in the lung.

As previously mentioned, there was no relation between right ventricular hypertrophy or hemoptysis and such pulmonary changes.

The lack of good correlation between organic pulmonary changes and the degree of mitral stenosis is provocative and has been discussed by us elsewhere.[14] It must

14. Ellis, L. B.; Bloomfield, R. A.; Graham, G. K.; Greenberg, D. J.; Hultgren, H. N.; Kraus, H.; Maresh, G.; Mebane, J. G.; Pfeiffer, P. H.; Selverstone, L. A., and Taylor, J. A.: Studies in Mitral Stenosis: I. Correlation of Physiologic and Clinical Findings, A. M. A. Arch. Int. Med., this issue, p. 515.

be remembered, however, that the present study was made from slides taken at routine post-mortem examinations and without a special investigation in mind, and that in almost all cases the only stain used was hematoxylin and eosin, which, as pointed out by Larrabee, Parker, and Edwards,[15] is less satisfactory for the determination of pulmonary changes than other stains for connective tissue. Moreover, in all but a few cases only one or two sections from lower lobes were available for study.

Larrabee, Parker, and Edwards[15] observed that organic changes in the blood vessels were present in 15 of 20 cases of marked mitral stenosis. Although it is impossible to compare our series directly with theirs because of certain differences in the technic of study and analysis, the proportion of patients with moderate or marked mitral stenosis in our series showing significant organic changes is roughly of the same order as that noted by these authors.

The lack of a better correlation between pulmonary changes and other demonstrably clear-cut clinical features, including the size of the mitral orifice, is surprising and of considerable importance in relation to the question of the selection of patients for operation. No definite conclusions can be drawn from this study,

TABLE 10.—*Age Versus Organic Pulmonary Changes*

Degree of Organic Pulmonary Changes	Total Patients	Age at Death		
		Below 40	40-59	60 and Older
None	22	0	6	16
Mild	37	11	18	8
Moderate	22	10	6	6
Marked	14	4	6	4
Total	95*	25	36	34

* Microscopic slides of the lungs of one patient under 40, of one patient in the group aged 40 to 59, and of four patients 60 yr. or older were not available for study.

of course, concerning the relation between organic changes in the pulmonary vessels and pulmonary hypertension, and it is the latter condition which probably governs the severity of symptoms. However, one can safely assume that many, if not all, of the patients with severe mitral stenosis suffered from some degree of pulmonary hypertension, and yet 65% of them had either no or mild organic changes in the lungs. Thus, pulmonary hypertension does not inevitably lead to pulmonary vascular disease. Because of the inadequacy of the methods of the present study, it is not possible to draw definite conclusions, but at least it appears highly probable that there are unknown factors determining the development of organic changes that as yet have not been elucidated. Gouley[16] also has observed this lack of correlation between pulmonary changes and passive congestion of mitral stenosis, and he suggested that rheumatic pneumonitis is the common denominator. Certainly further studies should be made from the point of view of deter-

15. Larrabee, W. F.; Parker, R. L., and Edwards, J. E.: Pathology of Intrapulmonary Arteries and Arterioles in Mitral Stenosis, Proc. Staff Meet., Mayo Clin. **24**:316 (June 8) 1949.

16. Gouley, B. A.: Evolution of Parenchymal Lung Lesions in Rheumatic Fever and Their Relation to Mitral Stenosis and Passive Congestion, Am. J. M. Sc. **196**:1 (July) 1938; Post-Rheumatic Role of Mitral Stenosis and of Post-Rheumatic Pulmonary Fibrosis in Evolution of Chronic Rheumatic Heart Disease, ibid. **196**:11 (July) 1938.

mining whether possible active rheumatic changes in the pulmonary circulation of the lungs may at some time be related to the degree of ultimate damage to the lung. In the present series specific pneumonitis was not noted in any case, and there was no correlation between the presence of active rheumatic myocarditis and the degree of change in the lungs and pulmonary vascular tree. This does not, of course, exclude the possibility that activity may have been present earlier.

ACTIVE RHEUMATIC MYOCARDITIS

All the microscopic slides of the myocardium were reviewed by one of us (S. R.) with respect to the presence or absence of active rheumatic myocardial changes. These were routine sections taken in all cases from the left ventricle or both ventricles and, in addition, in 21 cases from the left auricle as well as the ventricle. Although in such routine sections minimal changes of rheumatic activity may have been missed, it is unlikely that significant rheumatic myocarditis would have been overlooked.

The criteria on which the diagnosis of active rheumatic myocarditis was based were those established by Clawson.[17] While it is well recognized that acute rheumatic myocarditis may pass through many phases of activity from the acute exudative stage to the healed stage, only the typical proliferative stage with Aschoff nodules containing giant cells and collagen necrosis was considered as diagnostic of acute activity. Those cases showing suggestive collagen necrosis, focal lymphocytic infiltrations, and scattered macrophages were not considered as definite instances of acute rheumatic activity but were classified as consistent with the late or healing stages of active rheumatic myocarditis.

In three cases acute active myocarditis was seen, and in a fourth case the lesion suggested, but was not conclusive of, active acute rheumatic myocarditis. In six more cases the microscopic picture was consistent with late subsiding rheumatic myocarditis, although in three of these the evidence was not clear-cut. Thus, in a maximum of 10 patients of the total of 101, active rheumatic myocarditis may have been present on the basis of examination of routine sections.

In two further instances, focal interstitial myocarditis, not typically rheumatic, occurred. In one an acute myocardial infarction and in another a healed myocardial infarction were present. In one case, fibrinous pericarditis, not associated with definite rheumatic stigmas, was noted.

A review of the clinical records of these patients reveals the following pertinent facts: The three patients in whom definite acute active rheumatic myocarditis was seen were aged 19, 27, and 46 yr., respectively. In only one, the oldest of these, was active rheumatic fever diagnosed clinically, complicating his underlying chronic rheumatic disease, and it was believed both on clinical grounds and on the pathologic observations that the active rheumatic process materially contributed to his death. Of the other two, one died of pulmonary embolism after being admitted to the hospital because of a bleeding peptic ulcer, and the other was admitted to the hospital in congestive failure and was believed to have subacute bacterial endocarditis, although this was never established by positive blood cultures. The patient who was thought on pathologic grounds possibly but not conclusively to have active acute rheumatic myocarditis was a 24-yr.-old woman

17. Clawson, B. J.: The Aschoff Nodules, Arch. Path. **8**:664 (Oct.) 1929.

who had had congestive failure for 10 mo. She was admitted at the end of the eighth month of pregnancy, rapidly went into labor, was delivered, and died in congestive failure 12 hr. later. Five of the six patients in whom late subsiding rheumatic myocarditis was diagnosed were over 53 yr. of age; four of these were over 60. Of these, three died of cerebrovascular accidents, with no preceding cardiac symptoms of any severity, and two died in congestive heart failure. The sixth patient, 42 yr. old, died of occlusion of the mitral orifice with a ball-valve thrombus after six months of a progressively downhill course of cardiac decompensation and five years of mild congestive failure. Of the two patients with focal interstitial myocarditis, one was 51 yr. old and had subacute bacterial endocarditis diagnosed on both clinical and pathologic grounds. The other was 65 yr. old and died of a cerebrovascular accident.

Thus it is apparent that active rheumatic infection did not play an important role in the clinical course of the patients in this series. In the one case in which the rheumatic infection was a major factor in the terminal illness, its presence was suspected during life. In five other instances it was possible the acute myocarditis materially contributed to the death in congestive failure.

COMMENT

It is evident from the present study that mitral stenosis is an anatomic disturbance which may be surprisingly well tolerated by many persons for a long period and that its presence is often compatible with comparatively long life. On the other hand, it is equally obvious that ultimately almost all patients with mitral stenosis die as a consequence of their disease and that embolic phenomena are of great importance in determining the ultimate course and outcome. From clinical observation, however, there appears to be a group of patients with mitral stenosis who run a more fulminating or malignant course, characterized usually by severe chronic or recurrent pulmonary disability, manifested by dyspnea, acute pulmonary edema, hemoptysis, and chest pain in varying combinations. Such patients may die as a result of the direct consequences of such pulmonary failure, or they may go on to a stage of right-sided heart failure with peripheral congestion, or their lives may be terminated by embolism. Now that operation offers some relief to these patients, it appears to be important to sort out this group with malignant disease, if possible, from the larger group of persons with benign disease.

At present it is our opinion that operation should not be offered patients with mitral stenosis unless there is some evidence that the stenotic mitral valve is producing symptoms or other demonstrable proof of progressive breakdown in the circulation. It might be argued that the time to prevent future or irreversible difficulties from arising is at the stage before symptoms develop or before demonstrable strain on the pulmonary circulation or the right side of the heart. Against this point of view there are three strong arguments. The first is that shown in this study: In many instances the disease runs a benign course. The second is that it is reasonable to believe that surgical procedures for the relief of mitral stenosis will be improved in years to come and that, therefore, patients who are in a position to delay the procedure with reasonable safety for some years may have a much more satisfactory operation performed at that time. The third argument is that there is no clear understanding of the factors which operate to produce severe

mitral stenosis or of the time span involved in its development. We do not know whether the current operations on the mitral valve, which consist in the main of fracture of the fused commissures to enable better valve action, will be of permanent value or whether some inevitable trend of events which originally led to the stenosing of the mitral orifice will resume its action so that stenosis will once more recur, with the result that operation has only set the clock back for these patients by a few years. Obviously, patients now being operated upon must be observed for a great many years before this question can be answered clinically, and during this period it appears justifiable to select for operation only those patients who are already showing evidence of disability from their disease.

SUMMARY AND CONCLUSIONS

A report is made of the morphological observations at post-mortem study, correlated with the clinical findings, in 101 patients with definite stenosis of the mitral valve. Forty-eight of these patients were men and 53 were women. Fifty-eight of them were over the age of 50 when they died, and 38 were over 60.

Forty-seven of the patients had severe mitral stenosis, and, of these, 15 had either no or slight impairment of cardiac reserve up to the time of death or terminal illness. In 31 patients rheumatic heart disease was not diagnosed during life or only during the terminal illness.

Heart failure accounted for the death of 44 of the patients. Eight of these died as a consequence of subacute bacterial endocarditis. In only one patient was active rheumatic fever considered to be the direct cause of death. In 33 patients acute events in the peripheral circulation were the cause of death, of which 26 were cerebrovascular accidents. In the majority of cases emboli were responsible. Eleven patients died of pulmonary infarction, and in 20 more fresh pulmonary infarctions were present on post-mortem examination. Six died of pneumonia, and seven died of miscellaneous causes unrelated to the circulation.

Peripheral infarctions definitely occurred in 49 of the patients, and in 37 of these auricular fibrillation was present. In 10 infarctions were associated with bacterial endocarditis.

In 33 of the patients there were thrombi in the left auricle, and in 30 of these auricular fibrillation was present. In 26 of the 39 patients who had had recent infarctions and who did not have bacterial endocarditis, no thrombi were found in the left auricle at post-mortem examination.

Other factors concerned with the course of the disease and with the pathologic observations are also discussed, in particular, the degree of mitral stenosis in relation to objective findings, such as right ventricular hypertrophy. The significance of organic changes of the lung and pulmonary circulation is discussed; it was found that although, in general, these tended to be more frequent in patients with high-grade mitral stenosis, nevertheless, there was no exact correlation.

In 10 patients active rheumatic myocarditis may have been present, but in only one, or possibly two, cases did such active myocarditis appear to contribute significantly to the final illness.

The findings are discussed in relation to the question of the place of surgery of the mitral valve in the treatment of mitral stenosis.

Book Reviews

Diseases of the Heart and Circulation. By Paul H. Wood, O.B.E., M.D. (Melbourne), F.R.C.P. (London). Price, $12.50. Pp. 590, with 355 illustrations. J. B. Lippincott Company, 227-231 S. 6th St., Philadelphia 5, 1951.

This is a well-organized, new textbook which, in general, covers the material thoroughly and adequately. Specifically, physiological and anatomical principles are well discussed. Therapy is well outlined. The physical diagnostic aspects are well summarized. The more recent advances in cardiology are well covered, including congenital anomalies of the heart and related catheterization studies. The amount of material considered in electrocardiography, as such, is adequate for the need. Little space is given to the theoretical concepts and material which would not be of practical interest to the internist or general practitioner. In conclusion, one must certainly say that this book covers the ground very well and will be as useful to the internist or general practitioner as any other textbook of cardiology.

Primary Carcinoma of the Liver. By Charles Berman, M.D. Pp. 164, with 83 illustrations. Price, 35s net in Great Britain. H. K. Lewis & Company, Ltd., 136 Gower St., London, W.C. 1, England, 1951.

This is an unusual monograph which is particularly well written, printed, illustrated, and indexed. As Dr. Ernest Kennaway points out in the "Foreword," if the incidence of primary cancer of the liver were as great in any large white population as it is in the Bantu of South Africa, it would be held to constitute a major problem in cancer research.

The author, living in South Africa, has made a careful study of liver carcinoma as he has seen it among the Bantus, and he has done his best to compare the disease in that particular race with the disease as it occurs among other peoples. He has accomplished a scholarly piece of work which deserves high praise; his observations are well documented, and his conclusions are interesting and stimulating.

Conference on Connective Tissues: Transactions of the First Conference, April 24-25, 1950, New York. Edited by Charles Ragan. Price, $3.25. Pp. 164, with illustrations. Josiah Macy, Jr., Foundation, 565 Park Ave., New York 21, 1951.

It seems probable that if cortisone and corticotrophin (ACTH) had never become available this conference on connective tissues would not have been held. For practicing physicians, therefore, the section on the effect of corticotrophin and cortisone on connective tissues seems most important in view of the present great interest in collagen disorders. The various subjects are dealt with informally, the presentation being followed by round-table discussions which, in some cases, may be a little obscure. However, the Macy Foundation is to be thanked for making this material available.

Dimensional Analysis for Students of Medicine. By Harold A. Abramson, M.D. Price, single copy, $1; 6 to 25 copies, 75¢ a copy; more than 25 copies, 50¢ a copy. Pp. 41. Josiah Macy, Jr., Foundation, 565 Park Ave., New York 21, 1950.

". . . It is the pure scientists who now alone understand the use of our new and unpredictable weapons of hostility—such as the atomic bomb. The psychiatrists, as a whole, understand the mechanisms of unconscious motivation of hostility within man himself. Those who control the weapons of hostility and those who understand the psychomotive forces engendering hostility must meet on common ground as soon as possible. It appears to be a necessary condition that the 'pure' scientific disciplines and the psychological disciplines, antipodal in their very nature but meeting in the body of man, must merge if man is not to destroy himself."

The above paragraph taken from the introduction to this little book tells us its purpose, which certainly seems very reasonable. In the various chapters, however, such as "General Discussion," "The Circulatory System," etc., one immediately plunges into mathematical treatment which is

quite outside the scope of the reviewer's understanding and probably would be difficult for the average medical man. Thus, while the idea is excellent, we wonder whether in practice the result will be achieved with the average reader. Present-day medical students, however, better trained in mathematics, may find this point of view useful.

Conference on Problems of Nerve Impulse: Transactions of the First Conference, March 2-3, 1950, New York. Edited by David Nachmansohn. Price, $3. Pp. 159, with illustrations. Josiah Macy, Jr., Foundation, 565 Park Ave., New York 21, 1951.

This conference on problems of nerve impulse was sponsored by the Macy Foundation, which is to be thanked for its interest in promoting discussions on important subjects. Topflight authorities, under the leadership of Dr. Frank Fremont-Smith, have discussed the most modern and abstruse problems in regard to nerve physiology in vivid and telling fashion. This volume, however, is decidedly for the record and for specialists.

Emotional Factors in Cardiovascular Disease. By Edward Weiss, M.D. Price, $2.25. Pp. 91. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1951.

This is a small handbook which is well written and full of common sense. It is the kind of book that patients who are worried over their cardiovascular systems can read with benefit; physicians, too, can be guided by its philosophy. It emphasizes what doctors have known for centuries and too often tend to forget. Dr. Thomas Percival, in 1803, reminded us that "the *feelings* and *emotions* of the patient, under critical circumstances, require to be known and to be attended to, no less than the symptoms of their diseases. Even the *prejudices* of the sick are not to be condemned or opposed with harshness. For though silenced by authority, they will operate secretly and forcibly on the mind, creating fear, anxiety and watchfulness."

This booklet preaches from such a text.

1950 Year Book of Pathology and Clinical Pathology. Section on Pathology edited by Howard T. Karsner, M.D., LL.D. Section on Clinical Pathology edited by Arthur Hawley Sanford, M.D. Price, $5. Pp. 455, with 156 illustrations. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1951.

This year book abstracts those publications for the year 1950 in the fields of pathology and clinical pathology which seemed important to such experienced editors as Dr. Howard T. Karsner and Dr. Arthur H. Sanford.

The abstracts have been carefully prepared, are well written and well illustrated, and often are followed by short, pertinent editorial comments.

Three special articles are included which add to the book's interest: one by Hans Selye on the adaptation syndrome, one by W. Barry Wood Jr. on phagocytosis, and the other by Berta Scharrer on tumors in invertebrates.

The volume is small, readable, and admirably suited for quick reference. Its contents are about equally divided between pathology and clinical pathology. It deserves the popularity which it is almost certain to achieve.

Medizin in Bewegung: Klinische Erkenntnisse und ärztliche Aufgabe. By Richard Siebeck. Price, 27 marks. Pp. 520. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart O; agents for U. S. A.: Grune & Stratton, Inc., 381 4th Ave., New York 16, 1949.

In the preface the author states that the period of the great systems in medicine has passed. With firm intent he speaks of the clinical examination and management of the patient, rather than of the diagnosis and treatment of the disease. The contents of this clinician's book are based entirely on his personal experience, which obviously is large. He pays deserved tribute to Ludolf Krehl and Helmut Marx, former teachers and superiors with whom he was closely identified, especially the former.

The introductory chapter is a historical, clinical, and philosophical résumé of the factors constituting the basis of medical knowledge. The second chapter deals with disease and personality, in which he uses peptic ulcer as an illustration. The author observes that disease as gleaned from the life history always has many roots, internal and external, in the somatic as

well as in the physical sphere, in circumstances of personal and social nature. Chapter 3 consists of a discussion of the vegetative system. The thyrotoxicoses are given special consideration. In the opinion of the author reflection on Basedow's disease (toxic diffuse goiter) makes one realize what the vegetative system means to clinical medicine. Its unrest and irritability dominate the picture in this disease. The following 12 chapters deal with a variety of subjects, among which are metabolism, allergy, acute articular rheumatism, various acute and chronic infections, cardiovascular-renal disease, and carcinoma. A chapter is concerned with the psychosomatic and social factors in pathogenesis.

The volume contains much that is interesting and instructive. While great stress is placed on the psychosomatic, hereditary, constitutional, and other factors in pathogenesis, it is notable that the orthodox approach in treatment is still very apparent.

Klinische Chemie und Mikroskopie: Ausgewählte Untersuchungsmethoden für das medizinisch-chemische Laboratorium. By Dr. Lothar Hallmann. Price, 24.60 German marks ($5.85). Pp. 594, with 173 illustrations. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart O; agents for U. S. A.: Grune & Stratton, Inc., 381 4th Ave., New York 16, 1950.

This well-bound and well-prepared volume is divided into nine sections. The first section is concerned with the examination of sputum and stomach and duodenal contents; the second, examination of the feces (with good plates to illustrate the commoner parasites); the third, examination of vomitus; the fourth, examination of exudates, transudates, contents of cysts, and prostatic secretion; the fifth, examination of urine; the sixth, examination of spinal fluid; the seventh, examination of the blood (which is accompanied with colored plates); the eighth, normal values for blood, urine, and other body fluids (these pages are yellow and are thus easily located in the volume). The last chapter deals with various methods of evaluation, such as titration and quantitative chemical determinations of various body fluids.

This sixth edition is a good book which should be a real asset to the German physician or to anyone in this country who has a reading knowledge of German.

The Kidney: Medical and Surgical Diseases. By Arthur C. Allen, M.D. Price, $15. Pp. 588, with 1,200 illustrations. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1951.

This book is a good one. It is well illustrated with diagrams and photographs to correlate better pathologic and structural data with clinical expressions of renal function in the normal and abnormal renal states. Emphasis is placed on the functional pathologic viewpoint. Embryology of the kidney is presented briefly and lucidly, thus permitting a good discussion of congenital anomalies. The important renal diseases are presented, and each chapter is well documented. The printing is well done, and the photographs are clear.

Heparin und Heparinoide, Dicumarol: Möglichkeiten und Ergebnisse einer thrombostatischen und thrombolytischen Therapie. By Dr. T. Halse. Price, 13.50 francs. Pp. 240, with 41 illustrations. S. Hirzel Verlag, Claridonhof, Gotthardstrasse 6, Zurich, Switzerland, 1950.

The first chapter of this book deals with the history of heparin and its chemistry. It ends with a citation from Jorpes that so far it has been impossible to obtain pure heparin with well-defined chemical and physiologic properties. Attention is called to the relationship of the basophilic cells to the formation and storage of heparin. The action of heparin on coagulation is discussed. In association with a constituent of the plasma, heparin can inhibit the activation of prothrombin, but its main action seems to be that of an antithrombin. The formation of this antithrombin also requires the presence of an ingredient of the serum. The fibrinolytic activity of heparin is emphasized. A relatively simple colorimetric method for the determination of this activity is based on the faculty of the fibrin to adsorb carmine red, which is liberated on liquefaction of the fibrin. This fibrinolytic activity can be demonstrated in vitro and in the blood of rabbits after injection of heparin. It requires the presence of an intact "fibrinolysin," a ferment which is activated by heparin. Not all the commercial preparations of heparin evolve this activity, which seems to be of great importance clinically. The clinical use of heparin is discussed with reference to prophylaxis. Here the advantages and disadvantages of heparin, as

well as of bishydroxycoumarin (dicumarol®), are presented. The use of heparin for treatment of thrombosis and embolism stresses the immediate beneficial effect in cases of pulmonary embolism and the diminution of the danger of embolism in cases of manifest thrombosis or thrombophlebitis. Other indications for heparin therapy are given, as well as contraindications and its dangers.

The pharmacologic activity of a new heparin-like substance "thrombocid," a polysaccharide sulfuric acid ester, is discussed. The effect on coagulation was found to be mainly like that of heparin. But experiments in vitro did not show any fibrinolytic activity. Such activity could be readily demonstrated in the blood of animals and of man after administration of "thrombocid," which leads to an increase in the phosphatides of the blood, and these, in turn, favor fibrinolysis, as does lecithin.

The relation of the increase in phosphatides to fibrinolysis seems to require further elucidation. The fibrinolytic activity of "thrombocid" seems to become exhausted after a few days. Therefore, it is recommended that treatment start with administration of "thrombocid," to be followed after a few days with the use of bishydroxycoumarin to prevent further progress. This combined "thrombocid"-bishydroxycoumarin treatment proved beneficial in cases of thrombosis and of thrombophlebitis. It was given to more than 100 patients, of whom only 12 did not respond satisfactorily. Bishydroxycoumarin and its activity, as well as that of pelentan-tromexan,® is outlined. It is to be noted that tromexan® may not be so innocuous as has been claimed. The mechanism of the action of bishydroxycoumarin is discussed, and various methods for the estimation of prothrombin are described. The indications for treatment with bishydroxycoumarin, as well as the contraindications are presented. Finally, the morphologic and pathophysiologic foundations of thrombostatic and thrombolytic therapy are discussed. The literature is covered well. The book is to be recommended.

Klinische Physiologie und Pathologie. By Prof. Dr. Ferdinand Hoff. Price, 39 German marks. Pp. 782, with 124 illustrations. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart O; agents for U. S. A.: Grune & Stratton, Inc., 381 4th Ave., New York 16, 1950.

The introduction to this work sketches the development of the concept of disease through stages, such as humoral, organ, cellular pathology, and others. For convenience, a classification based on organ pathology is adhered to, even though this contradicts the concept of totality and is regarded as unbiologic.

The first major subdivision is devoted to the co-ordination of circulation, respiration, blood, and metabolism. Considerable emphasis is laid on antagonism between the sympathetic and parasympathetic nervous systems. It is to be regretted that the book does not concern itself more with the pharmacology of sympathicomimetic and parasympathicomimetic drugs which have been used rather widely to test the state of the autonomic nervous system. Fever and myeloid leucocytosis are given as indications of a sympathicotonic effect. Pyrifer (a bacterial protein preparation) is said to give the same effect, but the fact is not mentioned that fever and myeloid tendency do not always go hand in hand. A sympathicotonic effect following air encephalography is described several times, but without reference to the reasons for which this procedure was carried out, which may be important contributing causes of this effect.

The third main subdivision concerns lesions of the heart and circulation. The importance of study of peripheral capillaries is emphasized. Paroxysmal tachycardia is regarded as a simple illustration of the impossibility of connecting cause or causes with localized organ pathology. A consideration of the interrelationship of the vegetative nervous system, including its centers and the cortex of the brain, is needed. Reference is made to the combination of migraine and attacks of tachycardia. Migraine is connected with allergy which includes a central nervous system component among its fundamental features. Special interest, therefore, attaches to cases in which tachycardia occurred as an allergic symptom, such as has been described as following the ingestion of raisins. In this particular case, proof of the existence of a central nervous system component is hardly overwhelming. Some of the other clinical illustrations of the influence of the central nervous system are perhaps based too much on reason by analogy.

In the fourth main division, diseases of the respiratory organs are discussed, and in the fifth, diseases of the blood and of the blood-forming organs are considered. Diseases of the liver and bile passages form the subject of the sixth main division. It is stated that the occurrence of emotional icterus cannot be doubted. Reference is made to such an occurrence, which was ascribed to intense anger. But it is difficult to visualize anger as the sole cause, for many people

become intensely angry without resultant icterus. The author recognizes that such cases are very rare and not well understood.

Diseases of the pancreas are discussed in the seventh division and those of the gastrointestinal tract in the eighth. Theories of the causes of gastric and duodenal ulcers are given, and some truth is conceded to each.

Speaking of diseases of the kidneys and urinary tract in the ninth division, the author concedes that in cases of nephritis the renal lesions account for the symptomatology and the fate of the pateint. The extrarenal factors are not forgotten, and a special chapter is devoted to the extrarenal kidney syndrome. The statement that staphylococci, streptococci, or typhoid bacilli frequently pass from the kidneys into the urine without purulent involvement of the kidney needs substantiation.

Diseases of the organs of motion, bone, joints, and muscles form the subject of the tenth division. The eleventh division outlines the mechanisms of regulation. The unity of the organism is said to be the result of regulation. It is somewhat disturbing when the author speaks of life and death in terms of "regulation" and "cessation of regulation." What is meant probably is life and death of animals in which there is some degree of differentiation of the central nervous system. Plants in which the presence of a nervous system has not been proved may sicken and die or may recover. An ameba may die from the results of autochthonous changes, and the cells of tissue cultures may live and divide independent of any complicated extracellular regulatory apparatus. Here the author breaks a lance for the robust teleology which permeates the book. It is asserted that purposeful regulations take the leading part against the government of the living organism by the mechanics of cause and effect. It is not quite clear why refuge is taken in the uncertainty principle of Heisenberg, when links in the chain of cause and effect are not always readily discernible.

In a later chapter it is at least recognized that the data on which the concepts of unity, planning, and harmony and disorder rest are to be acquired by physical causal analysis.

The fundamental principles of regulation are (1) the principle of antagonism or of polar synergism; (2) the principle of interaction; (3) the principle of vegetative total commutation; (4) the principle of compensation; (5) the principle of functional circles, and (6) the vegetative rhythm. The functional circles constitute self-regulatory partial systems, as, for instance, the thyroid gland, by its hormone, acts on the diencephalon which influences cell metabolism and the hypophysis which, in turn, by its thyrotropic hormone acts on the thyroid gland.

The 12th division deals with the endocrine glands. It may be mentioned here that the molecular weight of insulin, which in this volume is given as about 35,000 actually is, according to recent investigation, about 12,000.

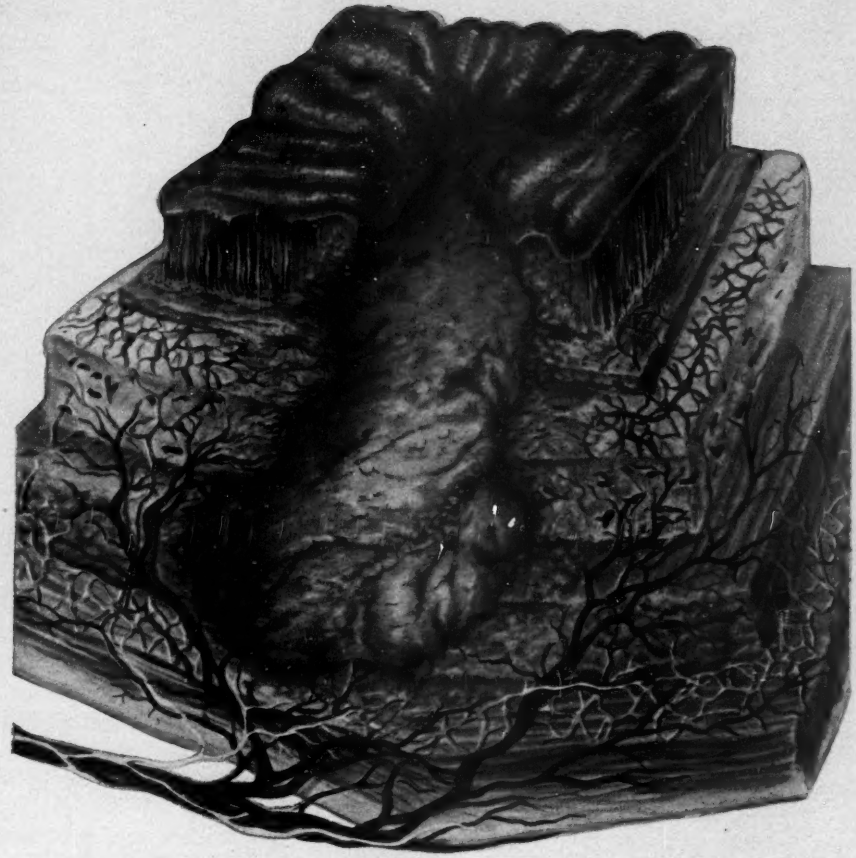
The 13th division deals with metabolism from the points of view of undernutrition, vitamin deficiency, and disorders of protein metabolism and of water and salt metabolism, disturbances of fat, lipid and carbohydrate metabolism, and of that of ketones and uric acid.

In the chapter on pathogenesis of edema it is admitted that in cases of nephritis without demonstrable diminution of oncotic pressure some tissue factor probably is operative. The author calls "erroneous" the claim that changes in oncotic pressure cannot be accepted as a cause of edema. Naturally, any force concerned with the distribution of water will be active under given conditions. But so far as overevaluation of any factor, such as that of oncotic pressure, is concerned, the fact that edema occurs without its diminution and that the edema fluid can be flooded out without change in this diminution, when it is present, cannot be dismissed so easily.

The existence of a hereditary or constitutional factor is accepted in explanation of a tendency toward adiposity or leanness. The author states that we know, for instance, in Westphalia and in Flemish Netherlands, that there is a stocky type of person who, although quite fat, is perfectly healthy; whereas in Frisia and in Sweden relatively lean persons are common. This manner of presentation may suffice in a popular novel, but not in a formal disquisition where such statements need better substantiation. Gout is regarded as a relatively rare disease which frequently is diagnosed erroneously, as is testified by the diagnosis of all possible rheumatic lesions as "gout." Gout is not so rare but that instances occur in which it is erroneously diagnosed as "rheumatoid arthritis."

The concluding remarks of the fourteenth division possess the virtue of relative brevity.

The book emphasizes an aspect of disease which has been neglected, and, since it contains much that is informative and stimulating, it can be recommended.



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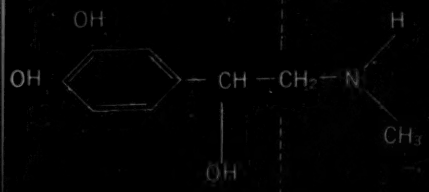

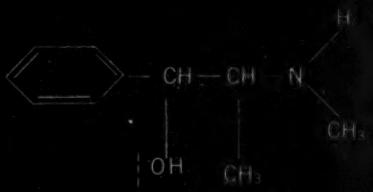

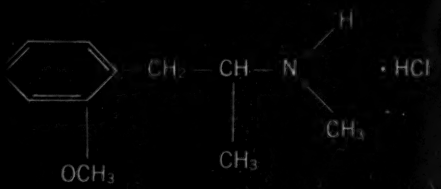

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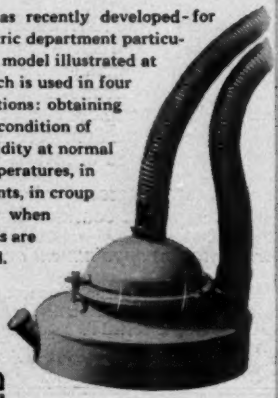

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RAHWAY, NEW JERSEY
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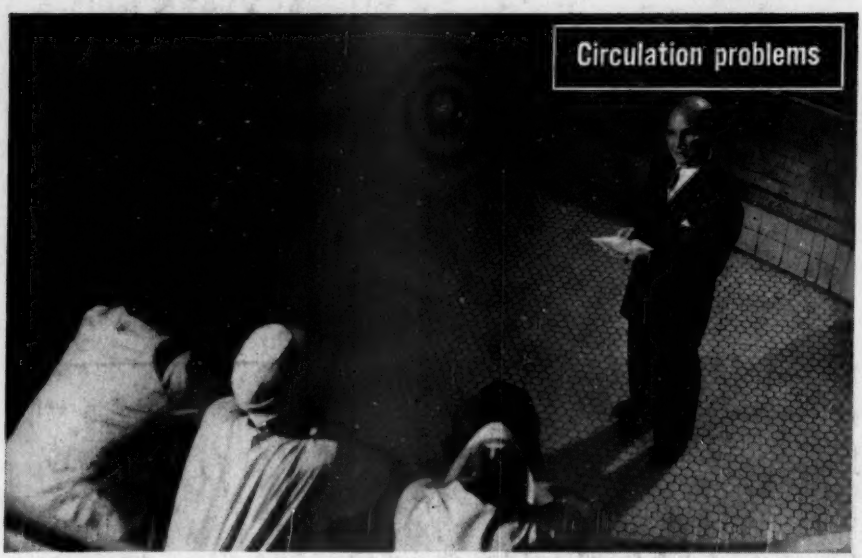
Cortone®
ACETATE
(CORTISONE Acetate Merck)



. . . and delay in replenishing the blood fluid makes it difficult to restore the original circulatory volume.

CONTROL of BLOOD VOLUME

Restoring Fluid Volume

Plasma infusions, and transfusions of whole blood, often make it possible to restore the circulatory volume satisfactorily without further ado. But under certain conditions it becomes difficult or practically impossible to achieve a satisfactory response without repeated transfusions or additional measures and continued treatment.

The problems of management are complicated by the fact that blood volume is difficult to measure accurately, and because there are no simple guides for determining what volume is "normal" for the individual to begin with. The problems are further complicated by the number of physiologic mechanisms involved, and our imperfect understanding of them.

Disadvantages of Delay

The *dangers* of delay in restoring blood fluid after conspicuous losses—when vascular collapse is imminent—need no further emphasis. Not so generally recognized is the fact that delay—in more common but less critical situations—actually makes it difficult to restore the circulatory volume to the original level. It would seem that failure to replace fluids promptly makes it difficult to do so at all, because the patient's "adaptive" processes adjust his physiologic functions to operate in accord with the new blood volume and, thereafter, attempts to increase the blood volume suddenly are "resisted." This indicates the need for replacing blood and plasma, volume for volume, as the losses occur.*

Lyophilized Plasma

Portable and stable without refrigeration, LYOVAC® Normal Human Plasma (Irradiated) is prepared according to the requirements of the National Institutes of Health. The plasma is pooled, irradiated to reduce the risk of homologous serum hepatitis, lyophilized and sealed under vacuum. It is supplied desiccated in vacuum bottles to yield 50 cc., 250 cc., and 500 cc. of irradiated normal human plasma (or smaller quantities of *hypertonic* plasma).

*Price, P.B., J.A.M.A. *145*:781 (March 17) 1951

Sharp & Dohme, Philadelphia 1, Pa.